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STUDIES ON THE OVERWINTERING OF PHYMATOTRICHUM ROOT ROT[1]

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INTRODUCTION

Though the cotton-root-rot disease, caused by *Phymatotrichum omnivorum* (Shear) Duggar, has been studied since 1888 (6), the development of successful control methods has been impeded by incomplete knowledge of the life history of the organism. The present paper includes the results of some studies on the means by which the fungus overwinters. In another paper (10), we have summarized experiments which indicated that the fungus spreads from plant to plant during the growing season, along the roots, which are the known avenues of spread. It appears from this previous work and the experiments reported below that living infected roots and sclerotia or dormant strands are the important means by which the fungus overwinters. So far as we know, it probably does not survive in the soil in a vegetative condition independently of roots.

I. OVERWINTERING ON INFECTED ROOTS

It has already been indicated (11) that infected live roots of susceptible hosts offer a ready means for the overwintering of root rot. Casual observers, even planters who have grown cotton for over forty years, have considered that, since the tops of the plants always succumb to the first killing frost in the fall, the roots are killed by the frost also. We have found that, although the tops of cotton plants are killed by frost, many of the roots of such plants remain alive through the winter. The root-rot fungus hibernates on roots apparently by gradually spreading during fall, winter, and spring and infecting the parts of the roots that were still normal at the time of frost.

The results presented below support the conclusion that *P. omnivorum* remains viable on infected roots in the ground only so long as such roots remain at least somewhat alive and apparently only in the vicinity of

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the living tissue. In these experiments, we have studied by various methods the viability of the fungus on roots of varying stages of decay. Such infected roots, to be tested in cultures as inoculum or in other ways, were in general classified or described as "living" or "decayed." We have classified as living all those infected roots with definite areas of live tissue irrespective of the amount present and including all stages from completely normal roots to roots nearly decayed but with occasional streaks of remaining live tissue. All other infected roots were classified as decayed, a group which therefore included roots with all tissues dead and also the occasional instances in which it was difficult to tell whether all the tissues were actually dead. Thus, infected roots were classified as living only when there was no doubt that such roots, though partly invaded by the root-rot fungus, contained large or small areas of living tissue; while the roots classified as decayed included those entirely killed by the fungus and also some roots in which, though the greater portion of the tissues was dead, there remained doubtful traces of live tissue.

Most cotton roots infected with root rot are readily grouped into the classes defined above because of the characteristic visible demarcation between the root-rot lesions and the noninvaded portions. The diseased areas are dark, softened, and sharply depressed; while the sound, noninvaded areas retain their normal color and turgidity. After the disease has involved the entire surface of the roots, there remains for a time some living tissue within the central cylinder. It was therefore necessary to cut into roots whose entire surfaces were decayed to find whether the decay was perhaps superficial only or extended to the center. This was done sometimes by sectioning with a knife or clippers or very frequently by thrusting the thumb nail into the tissues. By this test, we found it simple to differentiate the turgid, live tissues from the disorganized and softened decayed tissues. As noted above, however, doubtful infected roots were classified as decayed.

Winter survival of normal cotton roots.—In the springs of 1925, 1926, and 1928, taproots of normal cotton plants were pulled out to ascertain the condition of the roots after the winter. Table 1 shows that not only the taproots but also the laterals, and even many of the rootlets (Fig. 1, a and b) of the plants, had survived the winter, whether the plants were plowed under or left undisturbed. The viability of these roots was determined by visual examination, by cutting into the epidermis and cambium, and also by sprouting the roots in water or sand. Lateral roots survived in large numbers, probably because they were more deep-seated and therefore less likely to be disturbed by the plow.

Infection on overwintered roots.—The winter survival of taproots and lateral roots of cotton is significant here because such roots are frequently

TABLE 1.—*Overwintering of normal cotton roots, as shown by excavation and examination of roots in fields planted to cotton the previous season*

Date and location		Previous field treatment	Number of plants examined	Percentage of roots alive		
				Tap-roots	Large laterals	Fine laterals
1925						
March 7	Tyler	Undisturbed	200	80	85	49
"	"	Plowed under Nov., 1924	160	82	73	32
April 4	Brownsville	Undisturbed	146	100	98	92
"	"	Plowed under Nov., 1924	300	95	90	84
April 6	Temple	Undisturbed	500	83	70	41
"	"	Plowed under Dec., 1924	260	82	72	40
1926						
March 6	San Antonio	Undisturbed	600	96	93	92
"	"	Plowed under Dec., 1925	320	90	86	70
March 8	Laredo	Undisturbed	260	91	90	83
"	"	Plowed under Dec., 1925	400	91	87	74
March 17	Dallas	Undisturbed	300	80	73	65
"	"	Plowed under Jan., 1926	400	60	51	45
March 22	Wichita Falls	Undisturbed	259	60	53	47
"	"	Plowed under Jan., 1926	365	58	39	23
March 29	Balmorhea	Undisturbed	390	48	20	20
"	"	Plowed under Dec., 1926	451	40	23	19
1928						
March 6	College Station	Plowed under Oct., 1927	96	86	60	42
April 4	San Benito	Plowed under Sept., 1927	382	100	94	90
April 12	San Antonio	Plowed under Nov., 1927	465	91	80	71
April 14	Dilley	Plowed under Oct., 1927	260	100	91	68
April 16	Laredo	Plowed under Oct., 1927	325	92	100	100

infected with root rot. In table 2, observations are presented which show the frequent occurrence of root rot on the roots of cotton plants adjoining root-rot spots. Some of the plants found infected here doubtless were infected in fall, while others probably were infected as the result of winter spread, discussed below. Root-rot infection has similarly been found on the overwintered roots of many other perennial and biennial plants, non-cultivated as well as cultivated.

Viability of P. omnivorum on overwintered roots.—Cultures were made in attempts to isolate the fungus from infected material collected at different times of the year. The method of culturing was that previously described (8). The organism was readily isolated from cotton roots collected during the fall, winter, and spring months, though it will be noted (Table 3) that the percentage of pure cultures obtained diminished as the

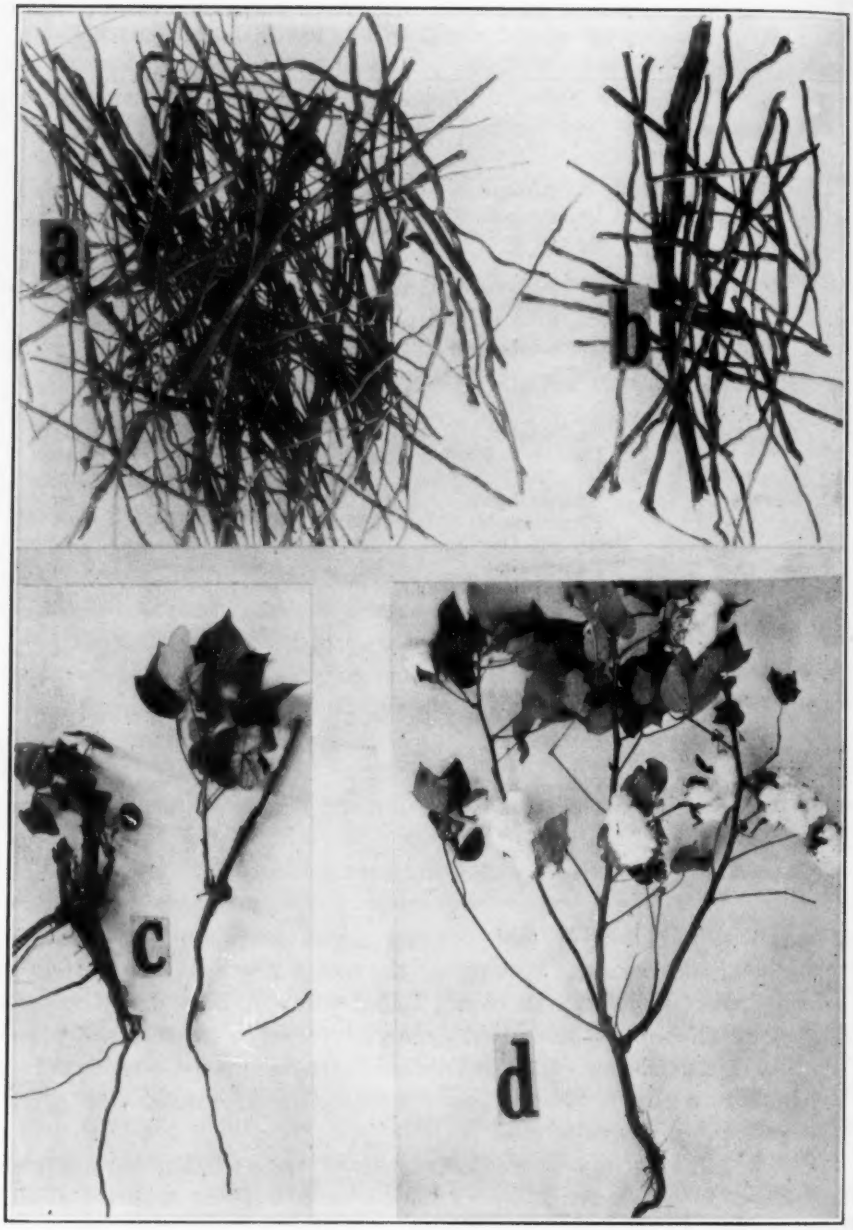

FIG. 1, a and b: Overwintered cotton roots found April, 1928, in a single excavation in a field at College Station; a, live roots; and b, dead roots. c and d: New growth from cotton roots overwintered in oat stubble field, Robertson County; c, sprouts from the overwintered roots; and d, mature plant, with cotton bolls, developed from overwintered root.

TABLE 2.—*Root-rot infection found in periodic field examinations of the living roots of cotton plants pulled from slightly beyond the periphery of root-rot spots*

Date and location		Number of plants examined	Percentage of plants with infected roots
Sept., 1926	College Station	46	12
"	Bryan	200	9
"	Benchley	79	30
"	Temple	300	28
Oct., 1926	College Station	160	21
"	Bryan	90	19
"	Benchley	150	17
"	Temple	300	24
Nov., 1926	College Station	25	16
"	Bryan	100	19
"	Benchley	100	21
Dec., 1926	College Station	100	14
"	Temple	300	17
Jan., 1927	College Station	100	9
"	Benchley	400	13
"	Temple	500	17
Feb., 1927	College Station	90	7
"	Temple	300	11
March, 1927	Bryan	150	9
"	Benchley	200	11
"	Temple	500	12
April, 1927	Bryan	100	6
"	Temple	200	9
May, 1927	Bryan	60	5
"	Temple	300	7

age of the material increased. The fungus was readily isolated from infected, living roots but not from roots which were infected but decayed.

Viability of P. omnivorum as compared with Fusarium vasinfectum, on dried roots.—Isolations have been reported previously (Texas Sta. Bul. 307, Tables 25 and 26) from infected cotton roots which were dried indoors and outdoors. Phymatotrichum could be obtained only from roots dried no more than twenty days. In contrast, isolations have been made from cotton plants infected with Fusarium wilt (*Fusarium vasinfectum*) which were exposed to air drying for various intervals. The results (Table 4) indicate that the Fusarium of cotton wilt, unlike *P. omnivorum*, is readily isolated from different parts of infected plants which have been dried for more than a year. We have also been able to isolate *F. vasinfectum* from

TABLE 3.—*Results of attempted isolations of Phymatotrichum omnivorum from living though infected cotton roots and from infected, decayed cotton roots*

Source of material	Date of culturing	Results with infected, living roots		Results with infected, decayed roots	
		Number of tubes	Percentage of tubes yielding pure cultures of *P. omnivorum*	Number of tubes	Percentage of tubes yielding pure cultures of *P. omnivorum*
Zemanek farm, Benchley, Texas	Aug. 16, 1926	90	12	45	0
	Sept. 22, 1926	111	7	316	0
	Oct. 18, 1926	390	6	316	0
	Dec. 14, 1926	518	4	260	0
	Jan. 16, 1927	400	2	100	0
Williams farm, Benchley, Texas	Aug. 18, 1926	210	11	210	0
	Sept. 24, 1926	464	6	209	0
	Oct. 20, 1926	416	5	210	0
	Dec. 20, 1926	414	3	270	0
	Jan. 18, 1927	200	2	87	0
	March 20, 1927	119	1	52	0
	Feb. 23, 1928	523	2	189	0
	March 22, 1928	427	1	136	0

infected cotton plants which had succumbed to the disease and overwintered in the field, while, as noted above, we have not been able to obtain *P. omnivorum* from cotton roots infected by root rot and decayed.

TABLE 4.—*Isolations of Fusarium vasinfectum from infected cotton plants exposed to air drying, beginning July 14, 1923*

Date of culturing	Age of material in days	Average number of Fusarium colonies per plate in culture from[a]		
		Main stem	Primary branches	Taproot
Sept. 12, 1923	60	25	23	.....
Oct. 11, 1923	89	.....	.....	29
Oct. 31, 1923	109	33	.....	32
Jan. 17, 1924	187	16	21	.....
Jan. 30, 1924	200	.....	.....	24
Feb. 15, 1924	216	9	.....	11
March 20, 1924	250	6	.....	9
July 17, 1924	369	2	.....	5

[a] The number of plates per series varied from 12 to 24.

Viability of P. omnivorum on infected live roots.—The isolation results in table 3 indicated that the root-rot fungus overwinters in an active form on live, infected roots but not on decayed roots. The following experiments, planned partly with the idea of developing a rapid method of determining whether the fungus was viable in roots of various stages of decay, have furnished additional information on this point. The first method tried consisted of collecting infected cotton roots, wrapping them in moist cloth, and placing these bundles of roots and cloth in various containers. Freshly infected living roots were compared by this method with older and with decayed roots. The results are given in table 5. With

TABLE 5.—*Relative production of new strands of Phymatotrichum omnivorum from living though infected cotton roots and from infected, decayed cotton roots; roots placed in moist chambers the day collected*

Root material		Treatment	Results after three weeks; development of *P. omnivorum* strands
Type	Number of roots and date collected		
Live, freshly infected cotton roots	23 roots, Dec. 11, 1927	Wrapped in moist cheesecloth and placed under covered bell jar	Profuse growth on 2 roots; fair growth on 19; scant growth on 2 roots
"	15 roots, Dec. 11, 1927	Placed in quart Mason jars two-thirds full of water, 3 roots per jar, 5 jars in all	Eight roots produced copious strand growth on the surface of the water. None on submerged area of any roots
Infected and decayed cotton roots	30 roots, Dec. 11, 1927	In quart Mason jars as above, except 5 roots per jar and 6 Mason jars in all	No growth
Live, freshly infected cotton roots	60 roots, Dec. 27, 1927	Wrapped in moist cheesecloth and placed in covered glazed crock	Profuse growth on 10 roots; fair growth on 10 roots; scant growth on 40 roots
"	100 roots, Dec. 28, 1927	"	Profuse growth on 60 roots; fair growth on 23 roots; scant growth on 17 roots
Infected and decayed cotton roots	1500 roots, Dec. 27, 1927	"	No growth

live, freshly infected cotton roots, the fungus grew out from the roots so abundantly that it was noticeable to the naked eye as well as microscopically. This occurred whether the roots were placed in moist chambers or in quart Mason jars with water. On the other hand, the roots that were

infected but decayed failed to yield any growth of *P. omnivorum* under the same conditions.

The question now arose whether the profuse growth of the root-rot fungus obtained from the cotton roots in moist chambers would be able to infect a susceptible host. To determine this, sound turnip roots were placed in moist chambers with live, freshly infected cotton roots; and other turnips were placed in contact with infected but now decayed cotton roots. Each series was set up within eighteen hours of the time the cotton roots were collected, and the roots were kept moist continuously until used. The results are shown in table 6. The sound turnip roots placed in con-

TABLE 6.—*Tests of the virulence of Phymatotrichum omnivorum from cotton roots to turnips, by wrapping in moist cheesecloth 12 sound turnips with each 100 cotton roots and placing the bundles in moist chambers*

Inoculum	Results after four weeks	
	Root-rot strands present on cotton-root inoculum	Percentage of turnip roots infected with root rot
Check. Live, normal cotton roots; 500 roots collected Oct. 25, 1927	None	0[a]
Live, infected cotton roots; 300 roots collected Nov. 1, 1927	Strands profuse on 60 per cent of roots; fair amount on 20 per cent; scant on 14 per cent; and absent on 6 per cent	68
Live, infected cotton roots; 500 roots collected Nov. 2, 1927	Strands profuse on 48 per cent of roots; fair amount on 50 per cent; and scant on 2 per cent	52
Infected, decayed cotton roots; 2,000 roots collected Nov. 5, 1927	None	0

[a] Turnips sound and sprouting.

tact with live, freshly infected cotton roots succumbed to root rot; while none of the turnips mixed with sound cotton roots or with infected and decayed roots were attacked.

AGE-OF-INOCULUM EXPERIMENT

To determine further whether the fungus *P. omnivorum* remains viable in the tissues of infected cotton roots after such roots are dead, inoculations of cotton plants were undertaken with inoculum consisting of the

roots of cotton plants which had succumbed to root rot at various intervals preceding the date of inoculation (Table 7). It has already been proved (8) that the roots from plants recently wilted from root rot can be used to inoculate healthy plants.

During the season of 1928, cotton plants in the experimental field at College Station were inspected daily for the first above-ground symptoms of root rot. Those plants that showed the characteristic sudden wilting were marked with a label that indicated the date. There were, therefore, available in September plants that had succumbed to rot rot quite recently

TABLE 7.—*Inoculations of normal cotton plants with root inoculum from infected cotton plants, pulled at varying intervals after the plants wilted from root rot. Each lot of inoculum included 20 taproots and was used to inoculate at least 20 cotton plants*

Lot No.	Description of inoculum pulled, and used in inoculations, September 13, 1928		Results of inoculations; percentage of plants infected with root rot, November 2, 1928
	Days since plants wilted from root rot	Abundance of Phymatotrichum strands on roots	
1	1	+++	92
2	7	+++	100
3	14	++	43
4	21	+	0
5	28	tr	0
6	35	tr	0
7	42	tr	0
8	49	tr	0
9	56	tr	0
10	63	tr	0

and had tops still green though wilted; other plants attacked during the middle of the season, with tops now dead but roots still alive; and, finally, plants that were attacked early in the season, with tops quite dried and roots almost completely disintegrated. Twenty plants were selected on September 13 for each of ten lots of inoculum. The intervals since the dates of wilting varied from one day for Lot 1 to seven, fourteen, etc., days by weekly intervals, to Lot 10 which consisted of roots from plants that had wilted nine weeks previously. The roots were removed from the ground after the soil around them had been loosened and care was taken not to lose the nearly disintegrated lower portions of the taproots of the more severely injured plants. In every case, however, the inoculum consisted of only the upper foot of the taproots rather than of the entire root systems.

Notes were taken on the state of preservation of these roots as pulled from the ground, before using them for inoculum in the inoculation series summarized in table 7. The more recently wilted plants had much the better-preserved roots. In Lot 1, the lesions had girdled the taproots but had involved only a small part of the tissues, most of which were still alive; and there was heavy mycelial growth over the surface of the roots. In Lot 2, the lower portion of the diseased areas had begun to disintegrate and the fungus strands there were deteriorating, although the upper portions of all the roots were still alive and bore the thick, fibrous strands typical of *P. omnivorum*. In Lot 3, the disease had progressed farther and involved the greater portion of each taproot, but each root had still considerable living tissue above the decayed area. In Lot 4, the surfaces of the roots were disintegrated and frayed; and the fungus threads were scarce, adhered now only loosely, and had lost their normal color. Apparently, the only tissues still living were those at the surface of the ground, and in four of the twenty roots even this portion appeared dead. In the root inoculum of Lots 5 to 10 there were further progressive decay of the roots and deterioration of the fungus. Living tissue in these roots was confined to the extreme top of the taproots, and the number of roots in which even this restricted region remained alive decreased progressively to 2 roots in Lot 9 and to none in Lot 10.

These selected taproots were used to inoculate large, normal cotton plants growing in metal cans of surface soil material of Lufkin fine sandy loam. The cans were twenty inches tall and fifteen and a half inches in diameter and contained about ten cotton plants, each. One of the pieces of cotton-root inoculum was used for each plant or occasionally for two plants. The plants were inoculated on September 13, 1928; brought into the laboratory October 30; and final notes were taken on the development of root rot on November 2, 1928.

As may be noted from table 7, there was in this experiment a definite relation of the interval between wilting of plants and the date when roots were used as inocula with the ability of this root inoculum to transmit root rot. Of twenty-five plants inoculated with roots from plants wilted the previous day, twenty-three became infected with root rot. All of the thirty-four plants inoculated with the week-old inoculum were infected, while only ten of twenty-three plants inoculated with two-week-old inoculum showed symptoms of root rot. Of the one hundred and twenty-seven cotton plants that were inoculated with taproot inoculum from plants that had wilted still earlier in the season, not one was attacked by the disease.

The fungus appears to have been virulent on the taproots of freshly infected plants for a period of between two and three week after the tops

of the plants had wilted. Further experiments during the same season (3) indicated that the interval between infection and wilting is often two to three weeks also. The total time between infection of roots of the cotton plants and the time when the fungus was no longer virulent on the upper part of the taproots was probably between four and six weeks.

It appears from this experiment that the root-rot fungus survived in an actively virulent condition only so long as some of the host tissues remained undecayed. It should be emphasized that the specific time of survival found here applies only to the particular seasonal and soil conditions of this experiment. Furthermore, it is to be remembered that only the upper foot of taproots was used as inoculum. This region is usually the first to be decayed, and, after the upper parts of the roots are dead, the root-rot fungus may still be spreading actively along lower roots which were infected later. The chain of events which includes infection, decay of the roots, and death of the root-rot fungus must of course occur much more slowly in winter.

PHYMATOTRICHUM OMNIVORUM ON THE LIVING PARTS OF ROOTS

Results of previous experiments (Tables 3, 5, 6 7), particularly in the isolation of cultures and the use of roots of diseased plants as inoculum in inoculation experiments, suggested that even on the roots of plants attacked by root rot the organism is usually present in a living condition only near parts of the roots as yet undecayed. Further test of this point was possible by a method in which portions of infected roots are thrust part way into moist, unsterilized sand in a moist chamber. In such soil chambers the root-rot fungus develops profusely from infected, live roots. This method appears quite valuable for determining the viability of Phymatotrichum on infected roots. On culture media, saprophytic organisms present in the rotted portions of the roots may smother and obscure Phymatotrichum growth. With the soil chamber method, on the other hand, while for a few days there may be a superficial development of saprophytic organisms, notably Rhizopus, this early, fluffy growth soon vanishes. The more slowly growing Phymatotrichum strands do not appear to be affected by this superficial contamination but grow at approximately the same rate and to about the same distance over the soil in the contaminated as in the uncontaminated chambers.

A preliminary series was started December 11, 1926, with a cotton plant collected in our experimental field at College Station. This plant had been inoculated with root rot some months earlier and only the upper part of the taproot was now alive. The entire root, from the dead, decorticated tip up to the normal part above the root-rot area, was sliced into pieces two cm. long, each of which was thrust into moist Norfolk sand in a pint

Mason jar. In ten days there was a profuse development of Phymatotrichum strands from the two pieces from near the junction of rotted and normal regions. One of these pieces had a live central cylinder with only the cortex rotted, and in the other piece not only the central cylinder but also part of the cortex was still alive. No root-rot strands developed from the lower portions of the root, where it was completely decayed, nor from the normal piece from above the diseased area.

On January 28, 1929, the taproots of six cotton plants of the 1928 crop were pulled from the edge of a root-rot spot in a field at College Station. These were naturally infected plants. The roots were found to

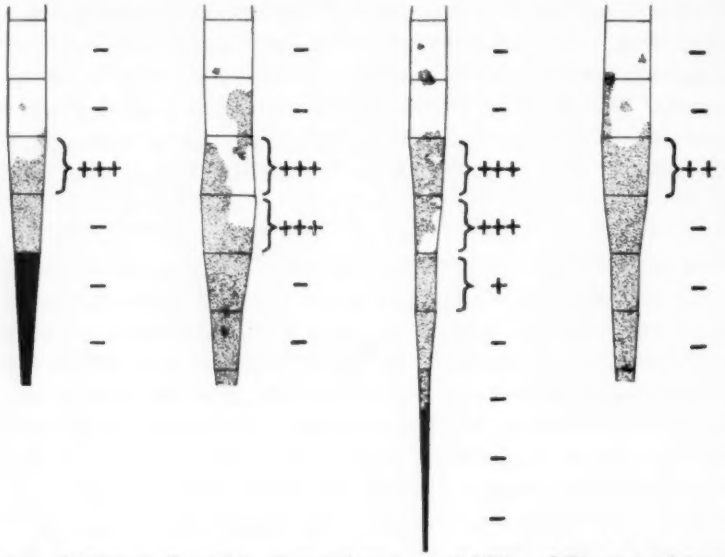

FIG. 2. Diagram of results of experiment on viability of Phymatotrichum in the living and dead parts of roots. In diagram, white areas represent portions of roots with cortex and wood alive; shaded parts, cortex decayed but woody cylinder alive; black areas, cortex and wood decayed. Roots cut into 2 cm. portions as indicated by horizontal lines; growth of strands from these portions when placed in soil chambers indicated by +, and no growth by –.

be at least partly alive, although all of them were definitely affected by root rot. They were taken to the laboratory, lateral roots were clipped off, and the taproots were washed gently in distilled water. Each root was cut into short pieces about two cm. long. These pieces were placed in moist sand in separate Mason jars which were then covered with inverted petri dishes. Observations on possible growth from the pieces were continued for five weeks. The results are presented graphically in figure 2, which, however, includes only the four roots from which some Phymatotrichum growth was secured. Profuse Phymatotrichum strands de-

veloped from sections of three of these roots and somewhat less extensive growth from one of the sections from the fourth. It is to be noted that in no case did growth come from the completely decayed portions of the roots. It was always from the sections at the junction of the normal and diseased areas. Six of the seven sections from which root-rot strands developed included both normal and diseased cortical tissues. In the seventh section the cortex was rotted but the central cylinder was still sound and was contiguous to normal cortical tissue.

These results indicate that *P. omnivorum* survives in an active condition only near the yet living portions of roots that are attacked by root rot rather than on the decayed portions of the same roots.

WINTER SPREAD OF ROOT ROT

As we have found that the root-rot fungus is active on live, infected cotton roots during the winter months, it was of interest to determine whether the fungus might also continue to spread along the roots after the tops of the plants are killed by frost.

Two cotton fields in the vicinity of College Station were selected in the fall of 1927 for these studies. A single large isolated root-rot spot in one field and two well-separated spots in the second field offered the opportunity of following spread that might occur. Initial notes were taken on September 30, when first all plants dead or dying from root rot were pulled and then the plants along each row were pulled until all those with taproots definitely infected had been removed. It was considered that the limit of the root-rot zone at the time was established when the final plant pulled had completely normal roots. The zones thus established (shown by solid lines in figure 3) included presumably all the plants infected during the summer and up to September 30.

Later observations were made October 27, November 30, December 20, January 22, and February 20. Notes for the two later dates could not be secured for one of the fields of figure 3, a, in which the cotton was plowed under early in January. At each date, cotton plants were pulled around the entire periphery of the spots until the limits of root infection were again established. The original area of the spots and the distances of spread were measured and are shown graphically in figure 3, a, b, c.

It is evident from these preliminary results that considerable spread of root rot occurred even during winter along these cotton roots. The longest spread appeared to have been in Row 10, figure 3, c. Here infection advanced twelve feet along the row between September 30, 1927, and February 20, 1928. It is to be noted that in the next row, 11, there was little advance of root rot during the winter. Similarly, in the other spot

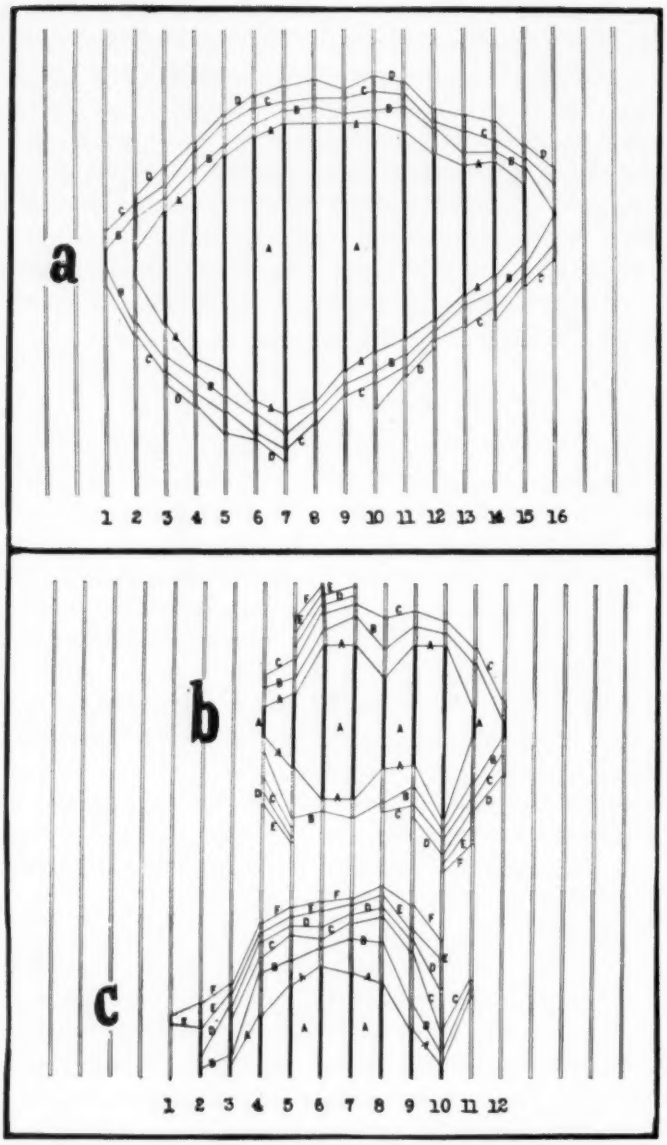

FIG. 3. Spread of root rot along cotton roots during winter; a, in field with a single large root-rot spot; b and c, in another field with two root-rot spots. Solid lines and areas included by line A indicate limits of infection on the roots on September 30, 1927; spread along roots during winter to B, by October 27; to C, by November 30; to D, by December 20; to E, by January 22; and to F, by February 20.

in this field (Fig. 3, b), spread in the upper part of Rows 4 and 5 was much more rapid than in Rows 2 and 3 during the first part of the winter; while, during January and February, spread continued in Rows 2 and 3 but ceased in Rows 5 and 6. These results indicate not only the extension of root-rot spots during winter but also seem to explain in part the apparently "erratic" reappearance of root-rot spots, which has been mentioned by many writers. These results further suggest that survival of *P. omnivorum* in an active vegetative condition during the winter months is the result of gradual spread of the fungus to previously normal roots or portions of roots.

Relation of weeds to overwintering of root rot.—Farmers often sow oats or wheat between the rows of cotton plants as soon as the cotton has been harvested. The land is usually not plowed, the grain being drilled

TABLE 8.—*Cotton roots, weeds*[a]*, and root rot on weeds found in stubble of 1927 oat field, that was in continuous cotton 1919–1927 (Cavitt Ranch, Robertson County, Texas)*

	Date of excavation	Percentage of live, overwintered taproots	Percentage of live, overwintered lateral roots
Live, overwintered cotton roots found in 6 excavations, 6 ft. x 6 ft. x 3 ft., at each date indicated	March 2, 1928	82	73
	May 2, 1928	42	21
	August 2, 1928	4	0
	September 1, 1928	1[b]	0

[a] The plants listed here were identified by Prof. H. Ness, late Botanist of the Texas Agricultural Experiment Station.

[b] The 1927 roots still alive on September 1, 1928, had all sprouted before this date and matured a crop.

	Names of weeds	Percentage root rot
Weeds growing in oat stubble and affected by root rot, September 1, 1928	*Sesban macrocarpa*	40
	Xanthium sp.	21
	Croton monanthogynus	17
	Sida spinosa	14
	Euphorbia humistrata	12
	Croton capitatus	7
	Aster exilis	7
	Eupatorium serotinum	6
	Iva ciliata	4
	Leptilon canadense	4
	Heterotheca subaxillaris	3
	Amphiachyris dracunculoides	3
	Jacquemontia tamnifolia	2
	Ambrosia psilostachya	2

in between the rows of cotton. As shown in table 8, this practice favors the overwintering of cotton roots (Fig. 1, c and d) and also affords a good opportunity for root rot to survive and to be transmitted to the numerous susceptible weeds which invariably appear on stubble land. While oats or wheat may be valuable in a rotation system, the stubble land should be plowed after the grain crop has been harvested and weeds kept down thereafter, if the rotation is intended to reduce root-rot damage to future crops.

Cotton and alfalfa growers in Texas have often noticed root rot appearing in the first crop on newly cleared land. Excavations were made to determine the source of infection in such fields, and the results are summarized in table 1 of Texas Bulletin 393 (9). Early infections in newly cleared fields planted to cotton were traced to infected native vegetation which persisted in the cleared land. Sclerotia may also have been present since they were not looked for in the excavations.

It is not uncommon to find roots of various perennials, particularly shrubs and trees, persisting in cleared land which has been cropped for years. The roots of such plants continue to sprout year after year, in spite of attempts to destroy them with the plow. This is the case with oaks, pecans, and native species of walnuts, all of which are susceptible to root rot. In excavations made at Lancaster, Texas, to determine sources of early infection in cotton, several pecan sprouts were found from stumps, which, according to the records of the owner, had sprouted every year for the last forty years. When excavated, many old root-rot lesions, in some cases healed over, were found on the pecan roots, and there were also lesions of more recent origin, together with strands of *P. omnivorum*. As will be discussed below, large numbers of sclerotia were also found in this field.

The studies above help to explain the persistence of root-rot in many fields. Cotton roots as well as those of many perennial weeds persist in the soil, even though the tops are plowed under during the fall or winter months, as uprooting the taproots and turning them under again do not necessarily kill them. The deeper roots are not reached by the plow and are probably even more important than the superficial roots in the overwintering of the fungus.

Inoculation of normal cotton plants with overwintered, infected cotton roots.—In order to demonstrate the virulence of *P. omnivorum* from overwintered, infected cotton roots, normal plants grown in the greenhouse were inoculated with root inoculum that had actually overwintered, undisturbed, in the field. This inoculum was secured in the lower Rio Grande Valley at Weslaco, Texas, and was pulled in a field of overwintered cotton

in which many of the plants had already sprouted and developed considerable new growth. Root rot had been present in this field during the season of 1928, and we found root rot on many of the plants on March 13, 1929. Infected taproots from overwintered cotton plants were taken to College Station and used to inoculate cotton plants growing in two large metal containers in the greenhouse. Although no wilting occurred in one container, some of the roots were found to be infected when the plants were removed at the end of the season; and all six of the plants in the other container became infected and died from root rot.

From the observations and experiments reported above, it is evident that one of the important ways in which *P. omnivorum* survives the winter is in an actively growing condition on infected but still living roots. The large number of infected but undecayed roots remaining in the ground no doubt offers an important source for early infections of the new crop.

II. SCLEROTIA AS MEANS OF OVERWINTERING

Early in the spring of 1929, numerous excavations were made, in cooperation with H. E. Rea, at San Antonio, Lancaster, Itasca, and Rockwall, to find the source of early infections in fields of cotton. In each place mentioned, the cotton was on land kept in clean fallow during 1928 but in cotton infected with root rot in 1927. With the exception of the Lancaster field, the excavations in 1929 showed that the fields were freed from most, if not all, of the roots of root-rot carriers. However, large numbers of sclerotia were found in excavation of the plants infected in 1929. This was particularly the case at Rockwall and Lancaster, where thousands of sclerotia were found under a single infected plant. It is probable that the sclerotia were produced on the infected cotton grown in the fields during 1927. Large numbers of sclerotia were also found in infected fields of garden beets, sugar beets, and sweet potatoes in truck gardens in the vicinity of Wichita Falls and Iowa Park.

King and Loomis (4) were the first to discover in pure culture the true sclerotia of the root-rot fungus. In the spring of 1929, Neal (5) found similar sclerotia in cotton fields at Greenville and at San Antonio. In the same spring of 1929, B. F. Dana, S. E. Wolff, and H. Dunlavy, all of the Blackland Substation, found sclerotia under infected cotton plants which grew on land which was kept in clean fallow during 1928. These observations agree with those of Neal and indicate that sclerotia are probably an important means of carry-over of root rot.

In addition to sclerotia, as mentioned above, Dana found what appeared to be dormant strands, separate or intermixed with the sclerotia. Similar strands were found by the writers in excavations at San Antonio, Itasca, and Lancaster, Texas. These strands are smooth and void of the outer

acicular fuzzy growth usually characteristic of strands of *P. omnivorum*. Occasionally, remnants of the fuzzy old acicular growth still remain clinging irregularly to the main body of the strand. When placed in moist chambers, the strands germinated readily at the broken ends, producing hyphae typical of *P. omnivorum*. Under field conditions, germination occurred from the exterior of any part of the strands. These dormant strands apparently function as sclerotia, which for some reason have failed to enlarge and develop into the typical shape. Both the sclerotia and the dormant strands were found at depths of eight to twenty-one inches in the ground.

Development of sclerotia in the laboratory.—King and Loomis (4) were able to develop sclerotia in cultures of *P. omnivorum* on sterilized sand and old cotton stems. Dana (1) found that Phymatotrichum growth from freshly infected carrots in moist soil in Mason jars produced large numbers of sclerotia. The writers have been able to develop sclerotia by using as inoculum pieces of cotton roots freshly infected with root-rot in glass containers filled with moist, unsterilized soil. With the method described above, using cotton or carrots as inoculum, sclerotia were produced in unsterilized surface soil material of Houston black clay, Bell clay, Susquehanna fine sandy loam, and Crockett clay loam. With soil containing 25 per cent moisture on a soil air-dry-weight basis, strands of *P. omnivorum* developed profusely along the walls of the containers in six to ten days. Within sixteen to twenty days, many of the larger hyphae began to swell at various places (Fig. 4), and these swellings matured into true sclerotia. The sclerotia are formed in chains or in compound clusters of irregular shapes. Individual sclerotia may be flattened or otherwise distorted to fit the space in which they are produced but are typically fusoid to ovoid. They are at first creamy to pearly white, then gradually turn darker, assuming finally the deep brown buff color of old strands.

In many of the containers in which carrots infected with root rot were used as inoculum, a profuse growth of *Sclerotium rolfsii* appeared as a contamination. The Sclerotium growth was obtained in jars in which Phymatotrichum strands and sclerotia were present as well as in other jars. The spherical sclerotia typical of *S. rolfsii* were produced in large numbers, and it was of considerable interest to compare these sclerotia with sclerotia of *P. omnivorum* produced under the same conditions. Our method of harvesting sclerotia from containers has involved washing, and it was found that true sclerotia of *P. omnivorum* are invariably heavier than water, while those of *S. rolfsii* are almost always lighter than water and may be separated by floating. It is, of course, easy to distinguish sclerotia of *S. rolfsii* by their smoothness and typically spherical shape. Sclerotia of *P. omnivorum*, on the other hand, are larger, irregular in out-

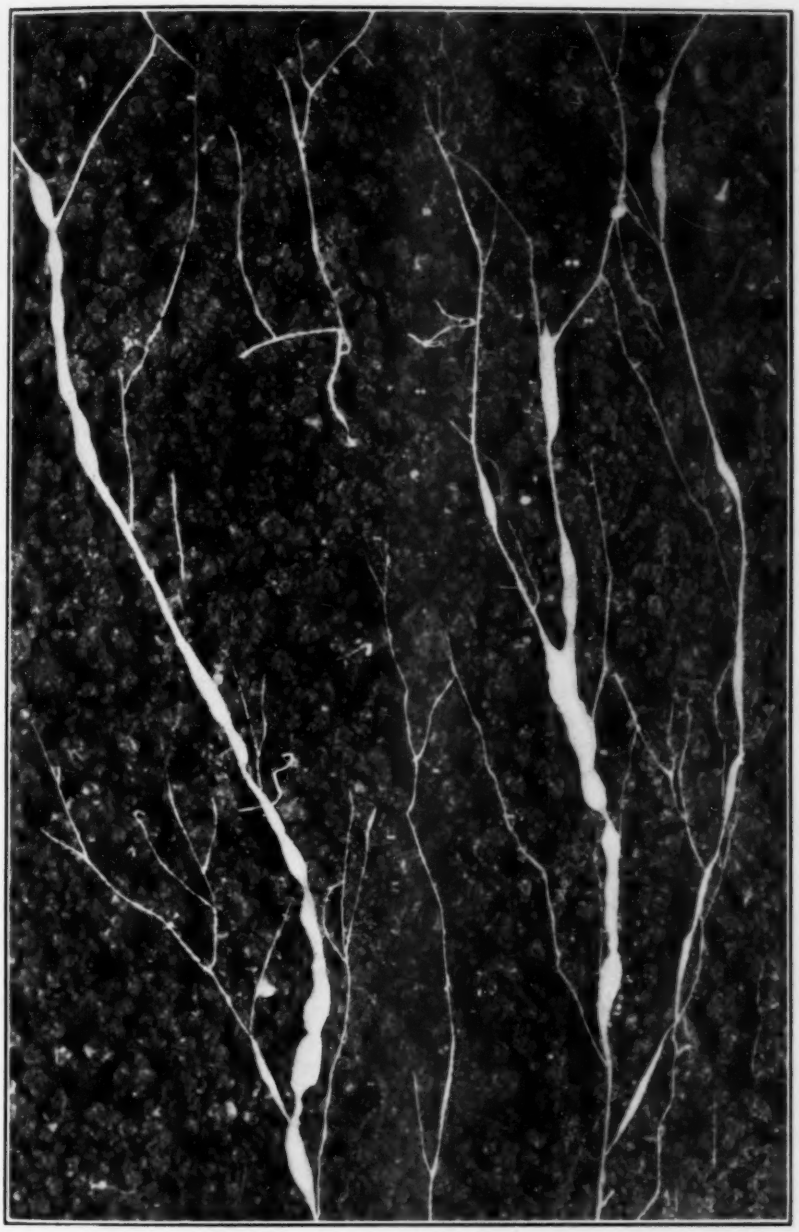

FIG. 4. Formation of sclerotia of *Phymatotrichum omnivorum* as swellings of strands developing in soil chambers. (Retouched photograph, strands, and sclerotia appear lighter-colored than was actually the case.)

line, and, unless mechanically disturbed, are always found in chains or masses or, if single, intercalary as swellings of the main strands.

Sclerotia were also produced in the laboratory at College Station in pure cultures of *P. omnivorum* grown on sterilized cotton stems and also in cultures on synthetic media. It is thus possible to obtain sclerotia of a definite age and from a known source. Results of these studies will be presented later.

Preliminary studies on the germination of sclerotia.—Sclerotia of *P. omnivorum* germinate readily in moist soil, on moist filter paper, or on agar. Germination of the sclerotia takes place at one or both ends of the sclerotium, and the resultant growth is typical of the root-rot fungus.

Three sets of sclerotia were used in one experiment: Sclerotia produced under carrots in a field at the Blackland Substation; some produced from carrot inoculum in glass containers in the laboratory; and a third lot produced from cotton-root inoculum in glass containers in the laboratory. These were washed out separately, placed on moist filter paper, on oatmeal agar, and on moist soil, and incubated in the laboratory at room temperature. All lots germinated rapidly under these conditions, producing yellow, branching strands. These were examined microscopically and found typical of *P. omnivorum*. It was noticed that sclerotia which failed to germinate were soft to the touch and crumbled with slight pressure, while the sclerotia which had germinated were plump and solid and did not crumble under light pressure.

Sclerotia may germinate more than once.—On October 3, 1929, a large number of sclerotia were tested for germination. Many of these produced profuse growth and still showed no evidence of deterioration at the end of the experiment. On October 17, the growth resulting from the previous germination was carefully removed from twenty-one germinated sclerotia, which were then placed again on moist filter paper. By November 4, thirteen of the twenty-one sclerotia had germinated, while the remainder failed to germinate and were found to have softened and in many cases become parasitized by various organisms, particularly *Fusarium* sp., *Phomopsis* sp., and a species of yeast. The growth from the thirteen germinated sclerotia was removed for the second time and the sclerotia placed again on moist filter paper. By December 9, 1929, four of these sclerotia had germinated and the growth was again clipped off. All four of these sclerotia then germinated for the fourth time. On January 3, growth was clipped again from these sclerotia and they were placed on filter paper. One sclerotium germinated the fifth time. The same sclerotia can evidently germinate at least five times under these conditions.

Pure-culture growth from sclerotia.—Sclerotia were germinated and transfers made to sterilized cotton roots and to various other media. The

growth resembled in every respect that obtained with isolations from infected roots. The strain isolated from the sclerotium has been grown on the various media on which stock Phymatotrichum cultures have been grown and is similar in cultural characteristics as well as microscopically. As detailed below, the cultures from the sclerotium as well as growth directly from sclerotia have been proved pathogenic to cotton plants.

Inoculation of normal cotton plants with sclerotia.—The following experiment was carried out to determine whether growth from germinated sclerotia can cause infection of cotton plants. Fresh sclerotia were washed out of soil obtained from a carrot field, dried to remove excess moisture, and placed at the bottom of quart jars, fifty sclerotia to the jar. Normal cotton plants were secured from a field free of root rot and placed in jars, which were filled with sifted surface soil material of Houston black clay to which was added 25 per cent moisture on a dry-weight basis. Pure-culture inoculum was used in other jars, and, finally, two jars were used as checks, which were left uninoculated. The jars were weighed daily and the original soil-moisture content maintained by adding water as required. Three days after the cotton plants were transplanted to the jars, the leaves of all the plants wilted, and within ten days all had shed. New leaves were later produced. When the plants were finally washed out for examination of the roots, clear-cut results were obtained, as shown in table 9. Infection of the roots occurred in every Mason jar where sclerotia were used as inoculum, and good infection also occurred where pure cultures were used. The uninoculated plants remained healthy.

Shortly after the beginning of the experiment, strand growth was observed on the glass walls of the inoculated jars. By November 16, many of the strands in jars a, b, c, d, g, and h of table 9, began to inflate into typical sclerotial forms, and, by November 27, the swellings of the strands had matured into sclerotia. At the end of the experiment, the soil in each Mason jar was washed carefully through a fine sieve and the residue examined for sclerotia. These were found in the jars that had contained inoculum, but, as was to be expected, in none of the uninoculated checks.

This experiment has proved that growth from sclerotia is able to infect normal cotton plants. Sclerotia of *P. omnivorum* probably play an important rôle in the overwintering of the causal organism. Considering the ease with which sclerotia may be produced in the laboratory, they may also serve as a convenient source of inoculum in experiments.

III. SPORE STAGES AS POSSIBLE MEANS OF OVERWINTERING

Phymatotrichum stage.—The Phymatotrichum, conidial stage of the root-rot fungus, was first described by Thornber (12, pp. 160–162) and was again described and named by Duggar (2). This stage appears as

TABLE 9.—*Inoculation experiment with sclerotia of P. omnivorum; inoculum placed on bottom of Mason jars which were filled with moist soil into which cotton plants were transplanted*

Inoculum	Jar	Results after two months	
		Condition of plants	Number of new sclerotia produced
50 Sclerotia per jar	a	One plant with top and taproot sound, but lateral roots infected; other plant, top dead, entire root system infected	124
	b	Both plants, tops dead, entire root systems infected	135
	c	"	140
	d	"	318
	e	"	0
	f	"	0
	g	"	157
Pure culture of *P. omnivorum* grown on sterilized cotton stems	h	One plant with top sound, taproot sound, only rootlets infected; other plant, top dead, entire root system infected	8
None (check)	i	Both plants, tops and root systems sound	0
	j	"	0

spore mats on the surface of the ground, usually around plants which have died from root rot. Spore mats are produced during warm, moist weather in the spring, summer, and early fall months. The writers have demonstrated actual continuity of hyphal growth from the subterranean Ozonium strands, with characteristic acicular branchings, to the conidia-bearing mycelium of Phymatotrichum spore mats aboveground (10): This, together with the fact that Taubenhaus and Killough (11) have found the Phymatotrichum spore stage in pure culture, leaves no doubt that the vegetative or Ozonium stage and the Phymatotrichum conidial stage are actually stages of the same fungus.

As yet, we have obtained only low percentages of germination with the Phymatotrichum spores, and the germ tubes produced soon disintegrate and seem incapable of further growth or infection. We do not know what

becomes of the millions of Phymatotrichum spores which are formed and are scattered by wind or irrigation water. There is no evidence that they can cause infection. We do not know whether the Phymatotrichum spores are a possible means of overwintering.

Possible perfect stage.—Shear (7) found a young sprout of Osage orange (*Maclura aurantiaca* Nutt.) dying from root rot. On the stem of the affected sprout appeared a hymenium with the typical spiny structure of a Hydnum. Believing this to be the perfect stage of the root-rot fungus, he named it *Hydnum omnivorum*. The evidence of a connection of the Hydnum stage with Ozonium, as stated by Shear himself, is only circumstantial. The writers have repeatedly found a Hydnum in cotton fields near College Station and in other parts of the State on the surface of the ground and on cotton plants previously killed by Phymatotrichum root rot. Tissue cultures were made from the spiny sporophores of this Hydnum, and the resultant growth has persistently failed to show any resemblance to Ozonium or Phymatotricrum.

In this connection, the writers have found a species of Coprinus also associated with roots previously infected by root rot. Sporophores of this fungus have appeared repeatedly on infected root material kept in moist chambers in the laboratory and on infected plants in the field. The possible genetic relation between this Coprinus and the Ozonium that causes root rot is yet to be determined.

SUMMARY

Phymatotrichum omnivorum, the cause of cotton root rot, has been proved to overwinter on infected live roots and as sclerotia in the soil. It may overwinter as spores but this has not been demonstrated.

Although the tops of cotton plants are killed by frost, the taproots, lateral roots, and rootlets may remain alive through the winter, and many survive until late the following summer. Root rot was present on roots found in field excavations during the winter and spring months. The viability of the fungus on overwintered roots was demonstrated by isolating the organism from the infected roots and by inoculation of sound turnips and cotton plants with overwintered, infected roots. *P. omnivorum* was viable on the living although infected roots but not on roots that were infected and decayed. This was in contrast with successful isolations of *F. vasinfectum* from cotton plants with wilt, even after the plants had been dried for a year.

In inoculations of cotton plants, infection was obtained with inoculum consisting of the taproots of plants which had succumbed to root rot only one day to two weeks earlier; while no infection resulted from inoculations with taproots from plants which had wilted from three to nine weeks pre-

viously. Infected roots were cut into sections 2 cm. long, which were placed in individual soil chambers. Strands from the sections which included living tissues but not from the sections of the same roots which included only decayed tissues. The disease not only remains viable on the living roots during the winter, but studies in two fields showed that root rot continued to spread during the winter, the maximum spread observed being twelve feet in one row. Root rot survives on the roots of many weeds as well as on cotton roots and may spread from these weed roots to succeeding crops. These results support the conclusion that *P. omnivorum* does not survive in an active condition on decayed roots but overwinters by continued though slow growth and spread on living, infected roots, and that overwintering on living roots is an important factor in the survival of root rot.

Excavations made in fields apparently free from overwintered roots but in which root rot was appearing on the new cotton plants revealed the presence of large numbers of sclerotia, which apparently were responsible for overwintering of the fungus in these fields. These observations confirm the work of King and Loomis and of Neal. Sclerotia have been produced also in soil chambers in the laboratory and in cultures on synthetic media. The sclerotia germinate readily and produce strands typical of *P. omnivorum*, and the same sclerotia may germinate at least five times under laboratory conditions. Cotton plants were successfully inoculated with a pure culture isolated from a sclerotium and with growth from sclerotia placed directly in the soil, and new sclerotia developed in these containers.

By dissection and microscopic study of Phymatotrichum spore mats from the field, it was possible to demonstrate actual continuity of the spore-bearing hyphae of the spore mats with the subterranean, typical Ozonium growth. Low percentages of germination have been obtained with Phymatotrichum spores, but no successful growth or infection from them has yet been secured. A Hydnum, frequently associated with plants killed by Phymatotricum root rot, does not resemble this fungus in pure culture, and definite connection of the Hydnum with the Phymatotrichum is yet to be determined. It is not yet known whether any spore stage is involved in the overwintering of the root-rot organism.

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FURTHER OBSERVATIONS AND EXPERIMENTS ON THE CURL DISEASE OF RASPBERRIES[1]

C. W. Bennett

For more than thirty years the disease generally known as curl has been recognized as one of the most common troubles affecting raspberries and, in the case of two or three of the more susceptible varieties, has been one of the most destructive diseases of this plant. Within the raspberry group there is a wide range of varietal susceptibility. The disease is widely distributed on the variety Cuthbert and occurs more or less extensively on other red varieties, such as Latham, Viking, and Marlboro. The Cumberland and Gregg are the most susceptible of the more popular black varieties. On red and black varieties symptoms of curl are practically identical under Michigan conditions, and this similarity has been tentatively accepted in the past as proof that the disease on both types of raspberry is caused by the same virus. Experimental evidence to confirm this view has not been produced.

Very few cross-inoculation experiments between red and black varieties have been reported. Smith (9) inoculated four black-raspberry plants from a red-raspberry source but obtained negative results. He attributed this failure to secure infection to the large size and lack of succulency of the inoculated plants and stated that the virus from the two sources probably would be found identical. In a previous publication (1) the writer has reported negative results from inoculations in which transfer from red to black varieties was attempted.

A further study of this disease under field conditions has been made during the seasons of 1926, 1927, and 1928. An excellent opportunity for extensive observation has been offered through the cooperation of representatives of the Orchard and Nursery Inspection Service of the Michigan State Department of Agriculture who, in the course of their work, have made two inspections each season of an average of more than a thousand acres of raspberries. Through the courtesy of E. C. Mandenberg, in charge of this service, records of these inspections have been made available for study whenever desired. Fields in which severe infestations have occurred, therefore, have been readily located in this manner and the development of the disease on all of the common susceptible varieties has been studied in fields rogued for control as well as in many fields where no measures have been taken to hold the disease in check. The information

[1] Most of the studies reported in this paper were made while the writer was associated with the Department of Botany, Michigan State College.

gained from these observations indicated that in 1925 the occurrence of curl on black varieties was very rare in the chief raspberry sections of Michigan. Many of the well-informed growers considered black varieties to be immune. Even at that time, however, in the plantings in which curl was known to occur, it spread rapidly and was recognized as a serious disease. Since 1925 the number of infected plants of susceptible black varieties has greatly increased in nonrogued fields and the disease has spread over a much wider area, embracing a large section in the southwestern part of the State.

Plants affected by curl in 1928 were found in a higher percentage of the fields of the black variety, Cumberland, than had been noted prior to that time. In most plantings only a few plants were infected, indicating a relatively recent introduction of the disease. This spread of curl in black varieties has seemed to bear no relation to the occurrence of the disease in the red varieties grown in the immediate vicinity. It is not at all unusual to find curl-free plantings of black raspberries which have grown adjacent to badly infested red-raspberry patches for a number of years.

Apparently, these field observations on raspberry curl are best explained by assuming that there are two distinct causal viruses which produce similar or identical symptoms. In 1925 inoculation tests were started to throw additional light on this question. These have been repeated and expanded each season until the evidence now seems fairly conclusive that we are concerned with two distinct curl viruses that thus far have been separated chiefly on the basis of varietal range of plants susceptible to infection.

For the sake of clarity, the terms alpha and beta will be used in referring to the curl viruses from the two sources. By alpha-curl virus is meant the one common in Michigan on the Cuthbert variety of red raspberry; by beta, the curl virus found in that State on the Cumberland variety of black raspberry.

The varieties used for most of the inoculations here reported are Cuthbert, Cumberland, and the purple or hybrid variety, Columbian.

ALPHA VIRUS FROM RED RASPBERRY PLANTS, TRANSMISSIBLE TO RED BUT NOT TO BLACK VARIETIES

It has been demonstrated many times that the virus of curl (alpha virus), common to red raspberries in Michigan, is readily transmitted by means of the aphid, *Aphis rubiphila*. Records of controlled experiments are available in which 230 red-raspberry plants have been inoculated from plants affected by this virus. Infection has occurred in 137 of these plants. During this same period, 115 black-raspberry plants have been inoculated

in the greenhouse or in cage experiments with virus from the same source, but none of these plants have shown signs of curl.

In the spring of 1926 a one-year-old planting composed of three rows (90 plants) of the variety Cuthbert and five rows (150 plants) of the variety Cumberland, both known to be very susceptible to curl under field conditions, was selected for a field test of the alpha-curl virus. Numerous aphids were reared under cages on Cuthbert plants affected by curl. These aphids were used to inoculate all of the plants in the field, an estimated number of more than one hundred aphids being released on each plant. The inoculations were made June 21. By July 30, fifty-one of the Cuthbert plants were showing marked symptoms of curl. They were employed to rear more aphids which were placed on the Cumberland plants. Again, in the spring of 1927, aphids were placed on the black-raspberry plants, also subjected to infection from natural migration of aphids from the Cuthbert planting. No curl developed in the Cumberland plants by the middle of the summer when ten of these plants were inoculated with the beta virus. Three of the inoculated plants became diseased. In the early spring of 1928, eighty-four of the Cuthbert plants were affected by curl and, of the Cumberland plants, only the three plants infected by the beta virus showed curl symptoms.

In February, 1928, an experiment was conducted to test thoroughly the alpha virus under greenhouse conditions on a number of red, purple, and black varieties of raspberry and on Lucretia dewberry. Ten potted plants of each variety (Table 1) were selected when about three inches high and subjected to the feeding of more than a hundred aphids. Five days later the aphids were killed by means of a spray of nicotine sulphate. After the plants had recovered from the effects of the feeding of these many aphids, they were again inoculated. This was repeated until five large lots of aphids had been placed on each plant. This experiment was performed in a greenhouse where curl on the variety Cuthbert was the only virus disease known to be present. As shown in columns one and two of table 1, all the Cuthbert plants became infected, while none of those of the black varieties showed signs of disease.

It may be noted that one of the plants of the red variety, King, became infected. This variety is very resistant to infection, although, after plants become infected, they show very marked symptoms of curl. During the five years in which curl has been under observation in Michigan only two affected King plants have been found, despite the fact that plantings of this variety are abundant in locations where they are subjected to chances of infection throughout the growing season. One of the Lucretia dewberry plants became infected also and leaf curling and stunting were severe.

Under field conditions no infected dewberry plants have been observed, and, so far as is known, this is the first recorded occurrence of curl on this type of cane fruit. Curl, however, does occur to a limited extent in fields of Eldorado blackberry.

BETA CURL VIRUS FROM BLACK RASPRERRY TRANSMISSIBLE TO RED VARIETY, CUTHBERT

Experiments in Michigan, extending over a period of four years, have shown that the type of curl peculiar to the black variety, Cumberland, is

TABLE 1.—*Results of multiple inoculation of varieties of raspberry and Lucretia dewberry with the alpha and beta viruses by means of large numbers of aphids, Aphis rubiphila*

Variety inoculated[a]	Alpha virus		Beta virus	
	No. of plants inoculated	No. of plants infected	No. of plants inoculated	No. of plants infected
Cuthbert (R)	10	10	10	4
King (R)	10	1	10	0
Columbian (H)	10	4	10	10
Plum Farmer (B)	10	0	10	0
Gregg (B)	10	0	10	6
Cumberland (B)	10	0	10	10
Lucretia Dewberry	10	1	10	0

[a] R = red variety; H = hybrid variety; B = black variety.

readily transmitted to healthy plants of this variety and also to Gregg. This virus also can be transferred to the red variety Cuthbert, now known to be susceptible to both types of curl virus.

To obtain additional information regarding the varietal range of the beta virus, an experiment similar to that outlined for red raspberry curl was made in which large numbers of aphids were placed on potted plants at five different periods of growth. Results of this experiment are presented in columns three and four of table 1. Of the black varieties, as indicated by this test, Plum Farmer is very resistant, if not immune. The variety Cuthbert, although susceptible, has consistently shown a lower percentage of infection than in those experiments in which the alpha virus was used.

After the Cuthbert variety was found to be susceptible to infection by the beta virus, transfers of this virus were made from infected Cuthbert plants back to Cumberland plants. The results of one of these series of experiments are given in table 2.

Here again, in the check plants of this experiment, it will be noted that no infection of black varieties occurred in inoculations in which the alpha virus was used. Infection of susceptible black varieties occurred in all cases in which the beta virus was used, regardless of whether the immediate source of inoculation was a red or a black variety.

TABLE 2.—*Results of cross inoculation of the Cuthbert and Cumberland varieties with alpha and beta viruses*

Varietal source of inoculum	Type of virus	Variety inoculated	No. of plants inoculated	No. of plants infected
Cuthbert	Alpha	Cuthbert	30	22
Cuthbert	Alpha	Cumberland	25	0
Cumberland	Beta	Cumberland	30	12
Cumberland	Beta	Cuthbert	30	8
Cuthbert	Beta	Cuthbert	30	13
Cuthbert	Beta	Cumberland	30	19

Cuthbert plants affected by the alpha virus were next inoculated with aphids from Cumberland plants affected by the beta virus. Two plants were used and large numbers of aphids applied. Transfers were later made from these plants to healthy Cuthbert and Cumberland plants. Other transfers were made as recorded in table 3.

In this series of inoculations, infection occurred on Cumberland plants in all tests in which transfers were made from Cuthbert plants which had been inoculated with the beta virus, regardless of whether the alpha virus was present. This test indicates that the Cuthbert plants affected by the alpha virus remain susceptible to infection by the beta virus and that the beta virus can be recovered from such plants. It seems that the alpha and beta viruses may thrive in the same plant for an indefinite period. This, however, cannot as yet be conclusively demonstrated experimentally, for, while the beta virus may be separated from a mixture of the two by making transfers to Cumberland plants, there is no means with the varieties used in these tests whereby the alpha virus may be separated from a like mix-

ture. However, a more complete study of the varietal ranges of susceptibility to these two viruses may demonstrate their separability by methods similar to those employed in the isolation of the virus of black-raspberry curl.

FURTHER EVIDENCE SUPPORTING THE VIEW THAT TWO DISTINCT VIRUSES ARE RESPONSIBLE FOR CURL IN RASPBERRIES

Regardless of the origin or possible initial identity of curl virus, it seems quite evident from the experimental work done that at present there are two viruses which differ with regard to certain symptoms manifested by inoculated plants. The origin of two such similar viruses from independent sources would seem less probable than a derivation of one from the other or both from a common source. If the latter hypothesis is

TABLE 3.—*Results of inoculations from plants having the alpha and beta viruses present separately and in combination*

Varietal source of inoculum	Type of virus	Variety inoculated	No. of plants inoculated	No. of plants infected
Cuthbert	Alpha	Cuthbert	10	7
Cuthbert	Alpha + Beta	Cuthbert	20	11
Cuthbert	Alpha + Beta	Cumberland	20	9
Cuthbert	Beta	Cumberland	20	12
Cuthbert	Alpha	Cumberland	20	0

accepted as the more probable, the justification for the assumption that the viruses are fundamentally different manifestly depends upon the stability of virus from red and black raspberry under different environmental conditions. The history of the introduction and spread of curl virus in Michigan, were it known, would probably shed light of fundamental importance on this problem. The rather complete separation of the two types of virus in the State probably is due to an early introduction of what is now the alpha virus on the variety Cuthbert and to the continued propagation of this virus for many years in sections where black varieties are rare or resistant to curls of all types. In recent years the introduction and extensive use of the curl-susceptible Cumberland have introduced a new factor into the curl problem. The first Cumberlands grown in the State probably were free from curl and remained so until the introduction of a virus capable of producing infection on such plants. The extreme scarcity of this

disease on black varieties, in 1922, and the rather rapid spread during the last five years, coupled with the fact that curl on wild black raspberry is extremely rare, indicate a relatively recent introduction. It has been generally accepted that curl has been more prevalent on black raspberries in certain parts of Ohio, Indiana, and Illinois, where black varieties are grown extensively, than in Michigan. With the interstate shipment of nursery stock the introduction of the beta virus from outside sources may have occurred a number of times. This may explain the occurrence, but rather limited distribution, of this virus in Michigan. It might be expected that the beta virus would be the predominant type on all susceptible varieties of raspberry in sections where red and black varieties have been grown for several years in the presence of the disease. It is probable even that the alpha virus would not maintain its identity under such conditions but would become mixed with the beta virus to such an extent as to be unrecoverable in a pure state by the known methods of separation.

The alpha virus has, in all probability, been present in Cuthbert plants in Michigan for more than fifty years. This long association with a single variety may have had a very decided influence in modifying properties of this virus. Carsner's (3) results in attenuation of the virus of sugar-beet curly top indicate that at least some viruses may be rather unstable and subject to modification under certain conditions of environment. The alpha virus may thus be merely a form of that peculiar to black varieties but changed sufficiently to influence its varietal range by its long association with the Cuthbert variety. All attempts, however, to modify either type of curl virus have failed to indicate that this may be easily accomplished. It seems convenient for the present to consider these as separate viruses, since a rather clearly defined distinction seems possible. This decision is reached, however, with the full recognition that further experimental results may render this position untenable.

The two curl viruses may be characterized briefly as follows:

Alpha curl virus. Produces severe symptoms of curl on the Cuthbert variety of red raspberry but does not affect black varieties. Causes very mild symptoms of curl on the hybrid variety, Columbian. Plants of this variety recover.

Beta curl virus. Produces severe symptoms of curl on the red variety Cuthbert, the black variety Cumberland, and the hybrid variety Columbian.

EFFECT OF BETA VIRUS MORE SEVERE THAN ALPHA ON THE HYBRID VARIETY COLUMBIAN

The Columbian is the most popular purple variety grown in Michigan and New York. It is believed to be a seedling of Cuthbert found growing

near a planting of the black variety, Gregg. This ancestry is significant since the Cuthbert is susceptible to both the alpha and beta viruses and the Gregg is susceptible to the beta virus only. Columbian plants affected by curl have not been observed in Michigan, although the variety has been under observation since 1922. It has been observed frequently in close proximity to Cuthbert plants affected by curl. Two instances were noted in which rows of Columbian plants grew within eight feet of and parallel to rows of badly curled Cuthberts for periods of three and five years, respectively, without contracting the disease. In July, 1928, however, a badly curled Columbian plant was found near Ravenna, Ohio. There were curled black-raspberry plants growing in the immediate vicinity and it is assumed that infection came from these plants.

Young Columbian plants have been inoculated with the alpha virus in seven series of tests. The plants have shown a mild form of leaf curl after twenty to thirty days. The margins of the leaflets curve downward and roll slightly inward. The plants are not stunted and the deeper green, characteristic of the disease in other varieties, is not, in most cases, evident. Under favorable conditions for growth, symptoms may soon disappear. In table 1 are recorded the results of one series of inoculations with the alpha and beta viruses in which large numbers of aphids were used and each plant was inoculated several different times. The plants from these tests were grown through the season of 1928, heeled-in in the fall, and planted in the greenhouse in February, 1929. The plants inoculated with the beta virus were very much stunted and showed severe symptoms typical of this disease on susceptible black plants. All of the plants inoculated with the alpha virus produced canes as stocky and vigorous as those produced by the healthy check plants. Mild symptoms of curl were evident on four of these plants. Figure 1 shows two plants of this series, one affected by the alpha virus and one by the beta virus.

The Columbian variety has been used as a source of inoculum in three experiments involving sixty plants, in an effort to transmit the alpha virus through a hybrid variety to Cumberland. All inoculations from Columbian and also those from the purple variety Haymaker have failed to produce disease in inoculated black plants.

RECOVERY OF COLUMBIAN PLANTS INFECTED WITH THE ALPHA VIRUS

In the case of the great majority of virus diseases plant pathologists are agreed that plants once affected rarely recover. Masking of symptoms, due to certain environmental conditions or to the nature of the plant involved, is, however, now known to be a rather common occurrence with a number of the mosaics. Complete recovery with loss of the virus is more rare. A few such cases have been reported.

Brierley (2) observed the production of apparently healthy shoots from a tomato plant that had been affected by mosaic but had lost most of its leaves. Inoculations from these shoots to healthy tomato plants failed to produce disease.

Verwoerd (11) has more recently reported a similar occurrence on tomato. Cuttings having mottled leaves were made from mosaic plants. In two instances cuttings developed into normal-appearing plants from which no infectious material could be obtained. It is not stated whether

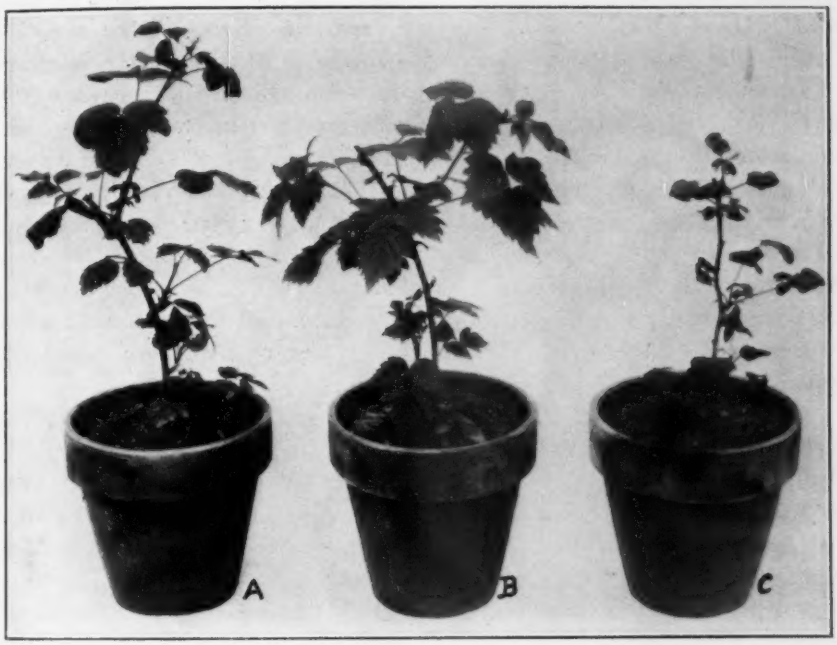

FIG. 1. Symptoms produced by the alpha- and beta-curl viruses on the purple or hybrid Columbian variety of raspberry. A, Columbian plant affected by the curl virus (alpha) from red raspberry. B, Healthy Columbian plant. C, Columbian plant affected by the curl virus (beta) from black raspberry.

these cuttings were tested when they were made to determine whether the virus was present at that time. It would have been interesting had this been done in view of the fact that Kraybill et al. (4) have shown that in one type of tomato mosaic there is present in diseased plants a separable fraction that will produce certain abnormal symptoms though apparently incapable of reproduction.

Recovery of certain varieties of sugar cane from mosaic has been found by Kunkel (5) to be a common occurrence. The tissues in the terminal

bud of the plants may recover and produce normal leaves after which the older, diseased leaves die and drop. In other instances the stools produce healthy shoots. Plants are subject to reinfection.

In transmission of streak from corn to sugar cane, Storey (10) found that the inoculated cane plants produced mild symptoms of disease and later recovered.

According to Severin (8), healthy branches occasionally are observed growing from plants of *Chenopodium leptophyllum* badly affected by sugar-beet curly top. Severin also found that inoculated plants of quail brush, *Atriplex lentiformis*, recovered from the effects of the curly-top virus. The plants after recovery were shown to be subject to reinfection.

In most varieties of raspberry there is no evidence of recovery from curl. Affected plants may live several years, gradually becoming more and more dwarfed until they die. In the case of the hybrid variety Columbian, however, there is evidence to indicate that plants regularly recover from the effects of the alpha virus after a period varying from a few months to two years and that the new growth, at least, is free from the virus. In the first series of inoculations with this variety made in 1924 very mild symptoms of curl were produced but the inoculated plants later seemed to recover. This apparent recovery was more extensively tested in 1925.

On February 20, 1925, ten Columbian tips growing in six-inch pots were inoculated with the alpha virus from badly diseased red-raspberry plants. Symptoms of curl developed on six of them. Transfers of aphids from each of these plants to five healthy Cuthberts produced curl in each lot of red raspberries inoculated. No curl virus was obtained from the four plants showing no symptoms. Five of the infected plants were transferred to the field in June and the sixth was transferred to a butter tub outside of the greenhouse. All of these plants showed curl symptoms throughout the summer of 1925. Transfers of aphids to healthy Cuthbert plants three different times during the summer showed the virus still present in the plant growing in the tub outside of the greenhouse. This plant showed mild symptoms throughout the season of 1926. In February, 1927, it was brought into the greenhouse where it made a vigorous and apparently normal growth. (Fig. 2.) Transfers of aphids from this plant to healthy Cuthberts no longer resulted in the production of curl. The plants in the field produced a very vigorous growth and no symptoms of curl were evident in 1926, the season following infection. Eight more Columbian plants were infected in February, 1926, and later were planted in the field. These showed symptoms of curl during that season but none were visible in 1927. Transfers of aphids in 1927, from these and from

the five infected plants placed in the field in 1925, produced no symptoms of curl on healthy Cuthbert plants.

The plant in the butter tub, which had recovered from infection with the alpha virus, was tipped and the tips as well as the mother plant re-

FIG. 2. A Columbian plant which was infected by curl virus from red raspberry February, 1925, and which recovered from the disease. Photographed April, 1928.

moved to Wooster, Ohio, in the fall of 1928. This plant and eight tips were planted in the greenhouse in February, 1929. Four tips and the mother plant were inoculated with the alpha virus. Infection was obtained in the mother plant and in three of the tips. The four remaining tips were in-

oculated with the beta virus and all developed marked symptoms of curl. Twenty tips from the plants inoculated in 1925 and 1926 and grown in the field also were selected and ten inoculated with the alpha virus and ten with the beta virus. Three and seven plants, respectively, became infected.

These plants, which had recovered from infection with the alpha virus, seemed no less susceptible to either the alpha or beta virus than plants which, so far as known, had had no contact with curl virus. The evidence available seems to indicate that the rate of growth influences speed of recovery, rapidly growing plants apparently recovering in a shorter time than those of less rapid growth. The recovery of rapidly growing Columbian plants from the influence of the alpha virus, together with the scarcity of the beta virus in regions where this variety is extensively grown, probably accounts for the fact that curl has been very rarely observed on plants of this variety.

FURTHER EXPERIMENTS IN THE TRANSMISSION OF CURL BY APHIDS

There are four species of aphids known to feed on the raspberry, namely, *Aphis rubiphila* Patch., *Amphorophora rubi* Kalt, *Amphorophora sensoriata* Mason, and *Amphorophora rubicola* Oestlund. The first of these was shown by Rankin and Hockey (7) to be a vector, and their results have been confirmed by a number of other investigations. Reports are meager regarding the other species. Smith (9) reported no infection in twenty plants inoculated with *Amphorophora rubi*. Bennett (1) also failed to obtain infection with this aphid in a limited number of inoculations. No one has reported inoculation tests with the two remaining species.

Since the species of Amphorophora consist of large and very active insects and since, if vectors of curl, they would be very important in dissemination, especially over long distances, it was thought worth while to conduct some rather extensive tests with all of those obtainable. *Amphorophora rubicola* has not been available in sufficient numbers for complete tests, although one series of inoculations has been made. In this experiment ten healthy Cuthbert plants were inoculated with five aphids, each. No disease developed on these plants, while, in the ten check plants on which *Aphis rubiphila* was used, five developed curl. The remaining two species have been used repeatedly and in large numbers. During the seasons of 1926, 1927, and 1928, 65 healthy plants have been inoculated with *Amphorophora rubi* and 35 with *Amphorophora sensoriata*. No curl developed on any of the plants inoculated. In March, 1928, ten healthy Cuthberts were selected and fifty specimens of *Amphorophora rubi* transferred from curl plants to each of the healthy plants. Five days after the

transfer these aphids were removed and fifty more were placed on each plant. Ten other plants were similarly treated, using *Amphorophora sensoriata*, and ten plants were inoculated with ten individuals of *Aphis rubiphila* per plant. Four of the ten plants inoculated with *Aphis rubiphila* became infected; none of those inoculated with *Amphorophora rubi* or *Amphorophora sensoriata* developed curl symptoms. These experiments were repeated in April, 1928, with essentially the same result.

Other experiments were conducted in which *Amphorophora rubi* and *Aphis rubiphila* were transferred from a Cuthbert plant having a combination of yellow mosaic, a medium type of mosaic, and curl. In one experiment *Amphorophora rubi* transmitted the two mosaics as a single virus to six of the ten plants inoculated, but no symptoms of curl developed. As a part of the same experiment, *Aphis rubiphila* was transferred from this plant to healthy Cuthberts with the result that four of the ten inoculated plants became affected by curl. None, however, showed any symptoms of mosaic. This experiment was repeated twice.

The beta curl virus was tested in a similar way on the Cumberland variety. A plant affected by curl and a medium type of mosaic was used from which to transfer the two species of aphids to healthy Cumberland plants. Here, again, in experiments in which *Aphis rubiphila* was used, curl was transmitted, and in transfers of *Amphorophora rubi* only mosaic was transmitted.

More experimental data should be available before any definite conclusions are drawn regarding the capabilities of *Amphorophora rubicola* for curl transmission. In the case of *Amphorophora rubi* and *Amphorophora sensoriata*, however, it seems reasonably certain that, if these species are capable of functioning as vectors of either of the curl viruses, they do so very rarely. It seems quite probable that neither of these species will transmit curl. Very little information regarding the underlying causes for the failure of these species to act as vectors is available. There is, of course, a possibility of a biological relationship involving *Aphis rubiphila* in the life cycle of a causal agent. However, if such is the case, experimental evidence indicates that the incubation period of the virus in the insect must be very short. Curl has not been transmitted artificially and indications are that the virus when outside of the plant is very sensitive to environmental conditions. It has been thought that this may possibly have a bearing on the apparent specificity of *Aphis rubiphila* in transmission in that this particular insect may be devoid of an inactivating substance which may conceivably be present in other species. This possibility as well as a study of tissues on which the different species feed would seem to offer fields for profitable investigation.

APHIS RUBIPHILA IN ALL STAGES OF DEVELOPMENT CAPABLE TRANSMITTING BOTH VIRUSES

In all of the earlier inoculation experiments with *Aphis rubiphila* the insects were used without regard to size of stage of development. After Rankin (6) reported transmission of mosaic on raspberries only with the younger instars of *Amphorophora rubi* and suggested that failure in his experiment to obtain transmission of mosaic by means of *Aphis rubiphila* may have been due to his using noninfective stages of the insect, it was determined to test the potentialities of different instars of *Aphis rubiphila* in the transmission of curl. It was found that aphids of this species molt so rapidly that it is difficult to keep accurate trace of instars where relatively large numbers of insects are used. For this reason plants were inoculated with aphids of different sizes, without regard to instars but representing progressively older insects from very young ones, 24 hours old or less, to and including wing forms, making the divisions small, medium, adult-wingless, and adult-wing. For these experiments, with the exception of the wing forms, colonies of first, second, third, and fourth generations from the stem-mothers were used. The aphids were reared on curl plants and removed from the inoculated plants after a period of twenty-four hours. Twenty aphids were placed on each plant with the exception of the plants inoculated with the wing forms, on which ten aphids per plant were placed. As shown in table 4, infection with both viruses was produced by aphids of all the sizes used.

TABLE 4.—*Results of an inoculation experiment with Aphis rubiphila in which insects of different sizes were used*

Size of aphid used	Cuthbert plants inoculated with the alpha virus		Cumberland plants inoculated with the beta virus	
	No. of plants inoculated	No. of plants infected	No. of plants inoculated	No. of plants infected
Small	10	2	10	3
Medium	10	4	10	3
Adult-wingless	10	5	10	6
Adult-wing	10	3	10	7

It has been demonstrated repeatedly that the wing form will transmit curl, and it seems quite evident that this form is very important in long-distance dissemination of this disease. During the seasons of 1927 and 1928 a number of instances of current or previous-season infection of

single plants were noted in fields which were being systematically rogued. In several instances the nearest known curl plant was not closer than a quarter of a mile and in two instances only one curl plant was discovered within a radius of a half mile. In sections where either of the curl viruses is present it should be expected that an occasional curl plant will be found even in fields which are carefully rouged. Such invasions of plantings from outside sources are very easily checked if affected plants are destroyed promptly.

SUMMARY

The curl virus, which has a general distribution in the Cuthbert variety of red raspberry in Michigan, apparently will not produce infection in the curl-susceptible Cumberland variety of black raspberries. The virus of curl which occurs on the Cumberland variety is transmissible to the variety Cuthbert and can be transferred from this variety back to black raspberry. It is assumed that two viruses are involved. These have been designated, respectively, as alpha-curl virus and beta-curl virus.

The purple or hybrid variety Columbian shows marked symptoms of curl when attacked by the beta virus and mild symptoms when infected by the alpha virus. Plants attacked by the alpha virus remain susceptible to infection by the beta virus.

Columbian plants infected by the alpha virus completely recover after a period varying from a few months to two years and new growth is free from the virus. Plants that recover remain subject to infection by both the alpha and beta viruses.

Transmission of the two curl viruses was obtained only by means of *Aphis rubiphila*. No curl infection was obtained in experiments in which *Amphorophora rubicola*, *Amphorophora rubi*, or *Amphorophora sensoriata* was used. All stages of *Aphis rubiphila* including the wing form transmitted curl. The wing form is considered to be very important in long-distance dissemination.

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SOIL-REACTION EFFECTS ON PHYMATOTRICHUM ROOT ROT[1]

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INTRODUCTION

It has long been noticed that cotton root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, is more serious in the black land regions of Texas, where the soils are alkaline in reaction, than in east Texas where many of the soils are slightly acid. A survey of fields in sixteen counties of Texas furnished preliminary evidence of a correlation between soil reaction and the occurrence and severity of root rot. The disease was found[3] in 34 per cent of fields with soils of pH 5.5 to 6.4, in 60 per cent of fields with soils of pH 6.5 to 7.4, and in 71 per cent of fields with soils of pH 7.5 and above. Furthermore, where root rot was found in acid soils, pH 5.5 to 6.5, damage from the disease was negligible as compared to the losses, ranging from 20 to 100 per cent, in the neutral or alkaline soils.

These results tended to show that root rot is destructive in neutral or alkaline soils but unimportant in acid soils. Experimental studies under controlled conditions were necessary, however, first, to prove that the correlation observed was actually with the soil reaction rather than with regional, climatic, or other factors, and, second, to find the critical soil reaction necessary to inhibit infection, to prevent spread, or to prevent overwintering of the fungus. The critical reaction may or may not be different for these various processes, and interruption of any one of these portions of the life history might serve to control root rot. The present paper includes summaries of some experiments which bear on this problem. Further work is in progress and will be reported later.

PRELIMINARY SOIL REACTION SERIES IN DRAIN TILES

In 1927 a series of 120 drain tiles, each 2 feet deep and 18 inches in diameter, were installed as containers for a preliminary experiment. The tiles were sunk in the ground, upright, nearly to the ground level, and filled with soil. Various materials were worked into the surface soil to change the reaction. Soil samples were taken periodically and the hydro-

[1] Paper read before the Des Moines meeting of the American Phytopathological Society and published with the approval of the Director as Contribution No. 96, Technical Series, from the Texas Agricultural Experiment Station.

[2] The writers are indebted to Dr. G. S. Fraps for advice in the planning of experiments and to Mr. W. T. Carter for identification of the soils studied.

[3] Taubenhaus, J. J., Walter N. Ezekiel, and D. T. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

gen-ion concentration determined colorimetrically. It was found that the soil reaction changed to depths of 6 to 8 inches only, the reactions obtained in this surface soil ranging from pH 2.3 to 8.0, while the deeper soil remained nearly neutral or slightly alkaline. For instance, samples taken in one container in May, 1928, ran pH 4.3, 4.3, and 4.4, respectively, for the upper three inches; 6.1, 6.5, 6.9 for the next three inches; and 6.9, 7.1, 7.1 for six to nine inches deep.

Cotton was planted in each container in 1927, 1928, and 1929. The plants were thinned to approximately an even stand and inoculated each year with root rot in every container. The method used was that previously described[4] in which roots of naturally and recently infected plants are inserted next to the roots of the plants to be inoculated. Root rot was introduced successfully, by this artificial inoculation, into every container irrespective of the reaction of the surface soil. There were differences in the numbers of plants attacked by root rot and differences in the numbers that died, in the various series. Summarizing all the results without regard to the treatment used to adjust the reaction of the soil, the results given in table 1 were obtained for 1928. It will be noted that there were higher percentages of infection of plants in the neutral and alkaline soils than in the more acid soils and that twice as high a percentage of the plants was killed in alkaline surface soils as in acid soils.

It is of particular significance that the disease overwintered in some of the containers in each series in the winter of 1927 and also of 1928, appearing on plants of the succeeding crop prior to the inoculation. Acidification of the surface soil alone then appeared insufficient to prevent the survival of root rot on roots in neutral soil below the acid region. At the same time, the high acidity obtained in the surface soil in many containers was enough to cause injury to plants and to prevent growth of cotton plants in the six containers of the most acid series in 1928 and 1929.

INOCULATION EXPERIMENTS WITH ALABAMA SOILS

Surface soil material of Houston black clay and Oktibbeha clay was secured in 1928 from Alabama through the courtesy of Mr. J. F. Stroud. Four cans were filled with each soil, cotton was planted, and the plants in each can were later inoculated with root rot. As shown in table 2, root rot was able to attack these plants growing in soils from a region in which Phymatotrichum root rot is unknown. The amount of infection and the percentage of loss from root rot were much higher in the more alkaline soil than in the only slightly acid Oktibbeha clay.

[4] Taubenhaus, J. J., B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk. A method of inoculation for Phymatotrichum root rot investigations. Phytopath. 19: 167–170. 1929.

TABLE 1.—*Results of preliminary series in drain tiles for 1928 season, considering containers with similar soil reaction together; the pH values apply to surface soil only, the subsurface remaining unaffected by the treatment.*

Average pH of surface soil, 0–8 inches	Number of plants included	Percentage of plants infected with root rot	Percentage of plants killed by root rot
2.9–3.1	0[a]	.....	.....
4.5–4.7	63	62	19
5.0–5.4	170	70	27
6.5–6.9	90	83	35
7.0–8.0	240	80	43

[a] On account of the excessive acidity in one series of six tiles, no stand was secured in 1928 or 1929.

INOCULATION EXPERIMENT WITH FIVE SOIL TYPES IN SMALL CONTAINERS

In the spring of 1928, an extensive experiment was set up primarily for the study of the incubation period and the development of root rot on the roots of cotton plants grown in five different soil types and inoculated at a definite time. Containers measuring two feet in each dimension were built with wood frames and with sides of wire screening and heavy tar paper. Eight containers were used for surface soil material of each of the five soil types listed in table 3. The containers were arranged side by side on the surface of the ground, and the spaces between and around them were filled in with soil to aid in maintaining natural temperature conditions. Cotton was planted, thinned to five plants; and, on August 14, two of the plants in each container were inoculated with two roots each from recently wilted cotton plants. One container of each soil type was opened at weekly intervals thereafter, the tops of the plants were secured by strings, and the soil was removed by washing (Fig. 1, c). The final series was held

TABLE 2.—*Results of inoculations of cotton plants growing in soil from Alabama; 20 plants per soil type*

Soil type	pH	Percentage of plants infected with root rot	Percentage of plants killed by root rot
Houston black clay	7.6	95	90
Oktibbeha clay	6.7	35	15

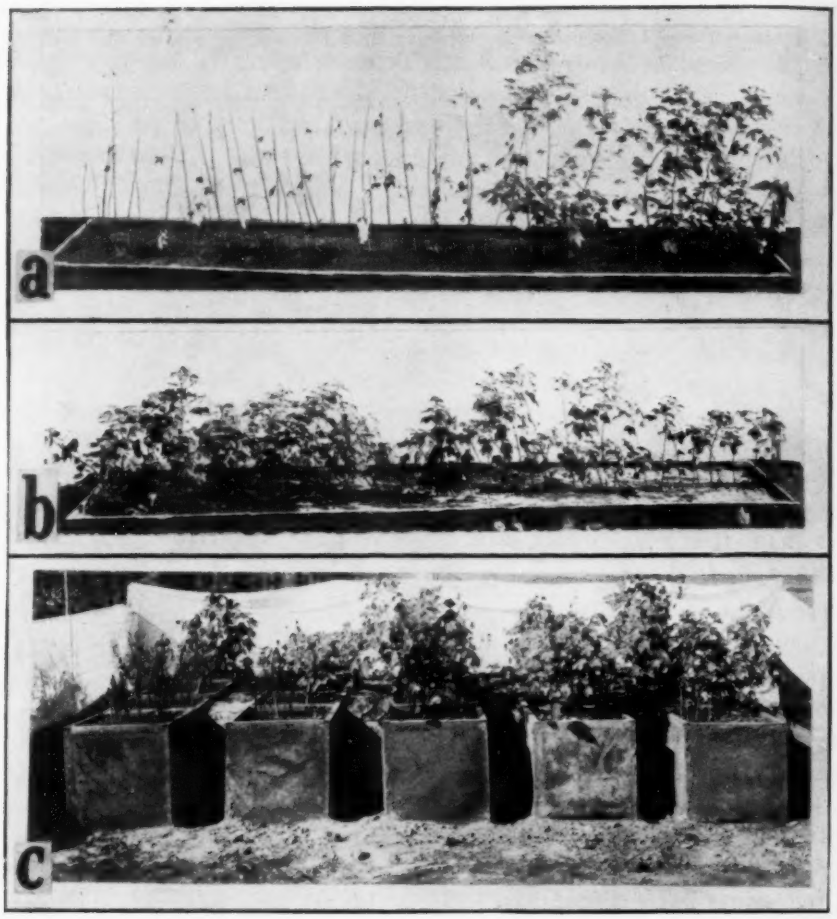

FIG. 1, a and b: Results obtained by November 15, 1928, from repeated inoculation at the left ends of the rows of cotton in boxes; a, of Houston black clay, pH 7.7, root rot spread more than halfway down the row; and b, of Tabor fine sandy loam, pH 5.8, no root rot.

c: Small containers of five soils used in experiment (Table 3) in which plants were periodically washed out. Photograph Sept. 18, 1928, showing surrounding soil already removed from around the five containers to be opened for final examination at that time.

until the spring of 1929, when it was opened and notes taken on the condition of the roots at that time.

The results for the first weeks were similar in the various soils. At the end of one week, there was no evidence aboveground of any injury to the inoculated plants. As the plants were washed out of the soil, the typical whitish to yellowish mycelial growth of the fungus was noted on

the roots of the inoculated plants along the portions of the roots adjoining the inoculum. After two weeks, there was still no evidence of the disease aboveground except for occasional plants which were freshly wilted, but

TABLE 3.—*Results of inoculation of 221 cotton plants grown in 8 containers per soil type and washed out at intervals after inoculation*

Soil type	pH	Average interval between inoculation and wilting of inoculated plants, days[a]	Percentage of plants with root rot, for entire experiment	Spread: Percentage of uninoculated plants with root rot found in washing out plants from fourth to seventh weeks after inoculation
Lufkin fine sandy loam	6.1	21.1	52	38
Crockett clay loam	6.2	19.5	58	67
Kirvin clay	6.4	19	68	81
Norfolk fine sand	6.8	15.6	63[b]	54[b]
Wilson clay	7.4	17	70	83

[a] Intervals calculated only from plants that wilted before time of washing out from the soil, and averaged for 10 plants each from series with Norfolk and Wilson soils and 11 plants from each of the other series.

[b] Figures include plants washed out after five weeks from the only container in the experiment in which the inoculation was apparently unsuccessful.

the mycelial growth on roots of the inoculated plants had extended over a more considerable portion of the taproots, and definite lesions were found on the portions of the roots first attacked. In three weeks, many inoculated plants had succumbed to the disease. Nine of the ten inoculated plants had wilted, and their roots were badly diseased, often to 30 or 40 cm. below ground. Lesions were beginning to be visible on the roots of plants adjacent to the inoculated plants.

By the fourth week, there were differences between the results in the different soil types. In the acid, Lufkin soil only the inoculated plants had become infected, while all five of the plants in the alkaline, Wilson clay were infected. Differences in spread were found also in subsequent examinations. As shown also by percentages in table 3, of the uninoculated plants examined from the fourth to seventh week after inoculation, ten of the sixteen plants from the Lufkin soil had escaped infection, while only two of the twelve uninoculated plants from the Wilson containers had escaped infection.

There were rather definite differences between the soil types with regard to the time elapsing between inoculation and the time when inoculated plants succumbed to root rot. In all types, some plants were observed to wilt by the twelfth, thirteenth, or fourteenth day after inoculation. The majority of inoculated plants had wilted by the end of the third week in all series. However, wilting of some of the plants in the acid soils was delayed longer than in the other soils, so that the average number of days between inoculation and wilting was greater for plants in the acid soils.

The final series of boxes was held until April, 1929. By this time, thirty-three weeks after inoculation, the roots of all plants in all five soils had become infected with root rot; and all were dead except for portions of roots in the Lufkin soil.

The Lufkin series was of further interest in that 6 of the 24 plants in the fourth to seventh harvests were affected by dark, gall-like excrescences along the taproots. These calluses resembled the effects of acid injury to cotton roots and perhaps resulted here from callusing following injury during cultivation. The calluses appeared of little importance in the growth of the plants.

This experiment showed that recognizable symptoms of root rot can appear on cotton roots in the five soil types used within a week after inoculation, while plants may wilt as a result of root-rot infection in 12 to 14 days in all the soil types, although, for the entire experiment, the average interval before wilting was longer in the more acid soils than in the more alkaline soils. Spread from inoculated plants to other plants in the containers was less in the more acid soil than in the other soils during the first seven weeks following infection, although by the following spring spread had occurred in all the soils. The most acid soil used here, pH 6.1, was not acid enough to prevent infection of inoculated plants and slow spread to adjacent plants.

INOCULATION EXPERIMENT WITH SEVEN SOILS, IN LONG BOXES[5]

In the experiments summarized above, the containers used were too small for detailed observation of the effect of soil differences on spread as distinct from initial infection. As has been reported elsewhere[6] an experiment was set up early in 1928 in which this difficulty was obviated by the use of larger, wooden containers 12 feet long, 3 feet deep, and 2 feet wide. Soils of seven different types were used, each in a single container. The soil material was dug out to a depth of three feet with each six-inch layer sacked separately and replaced, with each soil layer in its original depth, in the boxes at College Station.

[5] This experiment was installed by Dr. L. J. Pessin, formerly of this Division.

[6] Taubenhaus, J. J., and Walter N. Ezekiel. Recent studies on Phymatotrichum root-rot. Amer. Jour. Bot. 17: 554–571. 1930.

Cotton was planted in a row along the middle of each box in 1928 and again in 1929. During 1928, the plants at one end of each box were inoculated on June 22, August 8, and September 8. The results (Table 4) were in accord with the reaction of the various soils. Spread of root rot from the inoculated plants was successively greater in the soils with the higher pH values. No spread occurred in the most acid soils. Only one of the inoculated plants succumbed in the soil with pH 5.5 and not one plant in the Tabor soil with pH 5.8. (Fig. 1, a, b).

At the end of the growing season of 1928, the tops of the plants were cut off, but their roots were left undisturbed in the soil, and, on October 9, 1928, the roots of fifteen freshly infected cotton plants secured from nearby fields were inserted in each row to furnish additional material for possible overwintering. In 1929, cotton was planted again along the middle of each box without removing the roots of the 1928 crop. There was no inoculation in 1929.

TABLE 4.—*Summary of inoculation experiment with rows of cotton in long containers; inoculated repeatedly at one end of rows during 1928; no inoculation in 1929*

Soil type	pH of soil (0–2 feet deep)	Spread of root rot along rows during 1928, feet	Percentage of plants infected	
			1928	1929
Susquehanna fine sandy loam, shallow phase	5.5	0	2	8
Tabor fine sandy loam, shallow phase	5.8	0	0	0
Ochlockonee clay loam	6.3	4	27	59
Tabor fine sandy loam	6.7	4	29	34
Kirvin fine sandy loam	7.1	5½	30	80
Caddo fine sandy loam	7.6	7½	53	82
Houston black clay	7.7	7½	74	100

As shown in table 4, the disease overwintered successfully in every soil in which there had been any infection the preceding year, and in each soil it attacked a higher percentage of plants during the season of 1929 than in the previous year. The plants in the shallow phase of Tabor fine sandy loam were still free of root rot, while every plant in the Houston black clay was affected by September 12. Since the additional inoculum was inserted in 1928, it is possible that the increase of root rot in 1929 in some of the acid soils was due to this additional inoculum and that under field conditions root rot might instead have decreased.

While most of the soils used in this experiment were of approximately the same reaction to the depth used in the containers, the Tabor fine

sandy loam, shallow phase, in which no infection occurred either year, was distinctly acid only in the upper two feet and had a pH of 6.6 to 7.0 in the lower foot. This practically neutral subsoil apparently did not affect the results.

In the present experiment, the incidence and spread of root rot were found to correlate directly with the pH of soils held under controlled conditions. Root rot overwintered in all soils in which the disease had been able to infect cotton plants.

INOCULATION EXPERIMENT WITH DIFFERENCES IN DEPTHS OF ACIDIFICATION

In the spring of 1929, an experiment was started in which a single soil type, Lufkin fine sandy loam, was used in a series of 16 wooden boxes, 2 feet in each dimension. This soil was used in two ways, as indicated in table 5, either with 1 per cent of ground limestone added to the soil (on a soil dry-weight basis) or with the soil adjusted with sulphuric acid to pH 5.0. The amount of acid to use was determined with aliquot samples

TABLE 5.—*Inoculation experiment with cotton plants, six plants per wood box of Lufkin fine sandy loam; soil made alkaline by addition of 1 per cent ground limestone or acid by addition of sulphuric acid. Heavy inoculation, July 26, 1929. These results are of aboveground effect only, to end of season.*

Soil	Location of inoculum	Average interval between inoculation and wilting of inoculated plants, days	Number of plants succumbing to root rot
All alkaline (pH 7.2–7.3)	In upper foot of soil	20	6
	"	31	4
	In lower foot of soil	.....	0
	"	48	6
All acid (pH 4.9–5.3)	In upper foot of soil	.....	0
	"	.....	0
	In lower foot of soil	.....	0
	"	.....	0
Upper foot acid, lower foot alkaline	In upper foot of soil	78	1
	"	.....	1
	In lower foot of soil	.....	0
	"	.....	0
Upper foot alkaline, lower foot acid	In upper foot of soil	12	5
	"	21.5	6
	In lower foot of soil	.....	0
	"	.....	0

from the soil layers by the methods worked out by Fraps and Carlyle[7] for determining amounts of sulphur needed to adjust soils to desired acidities. In four containers, the entire two feet of soil was made alkaline, and, in another four, the entire depth was made acid; while four boxes were used with the top foot of soil acid and the bottom alkaline and four with the top alkaline and the bottom acid.

Cotton was planted June 11, 1929, and thinned July 26 to six plants per box, arranged in two short parallel rows. On July 26, the two plants at the same end of each row of plants were inoculated heavily with root rot. The inoculum consisted of the roots of freshly wilted cotton plants, trimmed down to exactly eight inches in length. Four roots were used for each plant inoculated. In two of each series of boxes, the inoculum was placed in the upper foot of soil by inserting it in holes bored to a depth of 10 inches with a soil augur; while in the other two boxes, it was placed in the lower foot of the soil in holes bored to 22 inches.

The results now available with this experiment are of the effects of root rot aboveground, since the roots have been left undisturbed. As shown in table 5, root rot killed most of the plants in the alkaline soil but none of those in the acid soil. Inoculation in the upper foot of the soil was markedly more sucessful than in the lower foot, irrespective of the reaction of these two soil layers. In the series in which the upper and lower layers were of different reaction, root rot appeared only where the inoculum was in the upper foot and in these boxes killed most of the plants in alkaline surface soil but only two of those in the acid surface soil.

The reaction of the acid soil selected on the basis of previous results appears to have been sufficiently acid to prevent inoculation with root rot under the conditions of this experiment. It did not interfere noticeably with the development of the cotton plants.

PRELIMINARY EXPERIMENT WITH SULPHUR FOR SOIL ACIDIFICATION[8]

The work referred to above indicated that root rot was more destructive and spread more in alkaline or neutral than in acid soils, whether this acidity was natural or obtained by acidification of the soil, and suggested the possibility of soil acidification for the control of root rot. The most economical and convenient method now available for acidifying soils in the field is to apply sulphur, which is slowly oxidized to sulphuric acid. This oxidation occurs more rapidly when the soil flora as well as other conditions in the soil favor oxidation. The present experiment was de-

[7] Fraps, G. S., and E. C. Carlyle. The basicity of Texas soils. Texas Agr. Exp. Sta. Bul. 400. 1929.

[8] This experiment was set up by W. F. Vogel, formerly Sulphur Research Fellow in this Division.

signed as a preliminary test of the possibility of reducing loss from root rot by the application of sulphur, and of the efficiency of eight different lots of commercial sulphurs, some of which lots included oxidizing materials. The results are combined below without regard to the various sulphurs used.

Surface soil material of Crockett clay loam was used in small metal containers some 10 inches and others 12¾ inches in diameter. There were four containers for each of the three rates of application of each of the eight sulphur materials. These materials were applied after the containers were filled, and were incorporated to a certain extent in the surface one or two inches of soil. The sulphurs were added June 12, 1928, and were still present in large lumps just below the surface as well as on the surface at the end of the season. It was apparent that, just as in the preliminary experiment in drain tiles (Table 1), the acidity resulting here was highly localized. This explained why the lower roots of some plants rotted from root rot, while parts of the taproots, closer to the surface, showed definite evidence of acid injury.

Cotton was planted and thinned to about four plants per container, giving a total of 14 to 16 plants for each application of each material and of 45 plants for the checks. Plants in all containers were inoculated with root rot by the usual method. Wilting of diseased plants was observed during the season, and at the end of the season all plants were pulled up for examination of their roots. It was found at this time that many of the dead plants in the containers had died apparently from acid injury, as evidenced by the characteristic enlarged cracked regions and the absence of Phymatotrichum strands or other symptoms of root rot. Plants with acid injury were more abundant in the series with higher rates of application of sulphur than in those with 5,000 lbs. per acre and were more abundant in the series in which the soil actually became highly acid than in the series of less acid soil.

Root rot, on the contrary, was less destructive in the more acid soils. Forty per cent of the check plants were killed by root rot, while only three per cent of the plants were killed in the series receiving 5,000 lbs. per acre, one per cent in the 10,000-lb. series, and none in the 15,000-lb. series.

The entire series of containers was held over winter and replanted to cotton in 1929. Despite repeated plantings, no stand could be obtained on account of the high acidity which had developed by that time.

In this preliminary experiment, excessive quantities of sulphur, confined almost entirely to the surface, reduced loss from root rot to a small fraction of the loss in the checks. There was, however, considerable acid injury to the plants, particularly in the most acid series. On account of

the uneven distribution of acidity, plants with the upper portion of the taproots injured by acid occasionally had the lower roots, extending into non-acidified soil below, destroyed by root rot.

FIELD TESTS OF SULPHUR FOR THE CONTROL OF ROOT ROT

A long-continued experiment with sulphur for the control of root rot was carried out at the former site of Substation No. 5, Bell County, with soil of a highly calcareous Bell clay. From 1920 until 1926, sulphur was applied yearly to various plats, in some cases at the rate of 10,000 pounds per acre. This did not exhaust the alkaline reserve even of the surface soil nor reduce the loss from root rot to a significant extent. Laboratory experiments by Fraps and Carlyle[7] have indicated similarly that it is impracticable to acidify highly calcareous soils by the application of sulphur.

Beginning in the fall of 1927, field tests[9] were carried on in seven localities in the State in soil types which are not highly calcareous and which accordingly might be acidified with quantities of sulphur that might be used in farm practice. These tests were initiated even before completion

TABLE 6.—*Inoculation experiment with cotton plants grown in small containers; results in the eight series with different sulphur materials combined here*

Sulphur added to soil	pH	No. of plants	Root rot		Sulphur injury	
			Percentage of plants affected	Percentage killed	Percentage of plants affected	Percentage killed
15,000 lbs. per acre	2.1–4.0	118	5	0	48	15
10,000 lbs. per acre	2.2–3.1	117	5	1	32	3
5,000 lbs. per acre	2.3–5.7	119	17	3	20	3
None (checks)	5.2–6.0	45	62	40	0	0

of the study of the field-survey results[3] which first demonstrated that the destructiveness of root rot in the field is correlated with the reaction of the soil. Empirical applications with various amounts of sulphur were made on the surface of the ground and incorporated somewhat into the surface soil. The tests showed that application of sulphur may acidify soils that are not highly calcareous; that excessive quantities of sulphur may cause acid injury of plants and may prevent obtaining a stand when the acidity

[9] These field tests were carried on by H. E. Rea, in cooperation with this Division.

TABLE 7.—*Results of sulphur experiment, data the same as in table 6, but arranged here by the average pH of the various series*

pH	No. of plants	Root rot		Sulphur injury	
		Percentage of plants affected	Percentage killed	Percentage of plants affected	Percentage killed
2.1–2.5	41	0	0	75	17
2.6–3.0	163	4	0	31	8
3.1–3.5	73	6	3	12	3
3.6–4.0	62	35	2	40	2
4.6–5.0	15	0	0	0	0
5.2–6.0 ..... (Checks)	45	62	40	0	0

becomes more intense than about pH 3.5; and that even so intense acidities as this will not control root rot if the subsurface remains alkaline and the disease survives there.

SUMMARY AND CONCLUSIONS

In a number of experiments, cotton plants were grown in containers filled with soils varying naturally in reaction and in other containers filled with soils in which the reaction was adjusted by the addition of various materials. Plants were inoculated artificially with root rot caused by *Phymatotrichum omnivorum*. The percentage of infection and the percentage of plants killed by root rot were higher in soils with the higher pH. Fewer plants were attacked in acid soils; the average interval between inoculation and wilting of plants was slightly longer in the acid soils; and root rot spread to a shorter distance as well as more slowly in acid soils than in neutral or alkaline soils. There was marked diminution of root rot at about pH 6.0 and no root rot with the soil at pH 5.0 to the bottom of the containers.

When only the surface layer of soil was acidified, without affecting the lower layers, the general correlation held true but was less pronounced. Root rot was reduced but still occurred in containers in which the surface was very acid while the subsoil was slightly alkaline. The disease also overwintered successfully in such containers.

Preliminary tests with sulphur for the control of root rot have been carried out in containers and in field plats. In both series, the sulphur was mixed only with the surface soil and acidified only the surface layer.

Root rot was reduced but not eliminated even with surface soil as acid as pH 3.4 when this acidity extended only to depths of three to six inches. Acid injury occurred in surface soils acidified to pH 2 to 4 even though the subsurface was still neutral or alkaline.

These results indicate the possibility of the use of sulphur for the acidification of soils as a method of attack against root rot but bring out some serious difficulties. Studies to determine the practical effectiveness of this method and to devise means of utilizing it under field conditions are now in progress and will be reported later.

STUDIES ON THE MOVEMENT OF THE CROWN-GALL ORGANISM WITHIN THE STEMS OF TOMATO PLANTS[1]

SPAS S. IVANOFF AND A. J. RIKER

INTRODUCTION

The movement which enables bacterial plant pathogenes to migrate from the point of entry into the surrounding tissue is of fundamental importance in relation to their pathogenesis. Once established within the host it appears that the further progress of the parasitic bacteria causing disease is influenced by the various factors which favor this movement. Since the movement of the bacteria through the tissue is important in relation to the development of the disease it seemed desirable to study the mechanism by which this was accomplished in connection with investigations on the crown-gall organism, *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B. The present paper gives an account of such studies on the movement of the crown-gall organism and certain other substances within the stems of tomato plants, primarily within a few hours after introduction of the bacteria through needle punctures.

Several workers of the last decade who have considered the crown-gall organism to be an intercellular parasite have dealt with this problem. Robinson and Walkden (7) report, "Both in *Chrysanthemum frutescens* and in *Nicotiana affinis* we have definitely demonstrated, by staining, zoogloeal strands of *B. tumefaciens* intruding along intercellular spaces and protoxylem vessels forming centers for pathological disturbance and gall-production along the tract." Riker (5) observed that, immediately following the puncture, a darkened area appeared, which tended to be parallel to the long axis of the plant and which was caused by the occupation of the intercellular spaces by liquid. He reports (6), "It seems unquestionable that the bacteria once inside such a wound would migrate to the limits of the avenue provided by the flooded intercellular spaces." Also he says (5), "The forces which govern this entry of the organism into the tissue are not definitely understood. The bacteria might conceivably be influenced by any or all of such factors as the collapse of drying tissue, negative pressure, sap rise, and motility with or without a chemotactic

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

These studies are a part of the work on the crown-gall project which is being carried on in cooperation with the United States Department of Agriculture.

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stimulus." Hill (3), following the method of investigation similar to that employed by Nixon (4), by Beach (1), and later by Haber (2), reaches these conclusions: "It is evident from my observations of the early stages of the migration resulting from inoculation that, after a few hours following their introduction into the tissues, the bacteria can be recognized only as widely scattered clumps clinging in the intercellular spaces. This is due to the fact that the rate of growth is very rapid and that after a few hours the tips of the zoogloeae pass through the tissues leaving occasionally a few scattered clumps of bacteria. . . . The rate of migration of *B. tumefaciens*, or actually the rate of growth of the zoogloeae of this species, is so rapid that very early stages can be observed only in material killed within one hour after the bacteria have been introduced into the tissues. . . . The rate of growth of the zoogloeae through the intercellular spaces is from 0.029 mm. to 0.04 mm. a minute, the more rapid rate occurring in the early stages of the migration following the introduction of the bacteria into the young tomato stems. The rate is most rapid for the first 30 minutes following inoculation. After three hours the rate is reduced one half."

The difficulty of demonstrating the crown-gall organism within the host tissue following its introduction through punctures has been an obstacle to all the investigators of this question. The characteristic staining properties of this organism are so nearly those of the host tissue that an individual organism or even a small group of several hundred organisms might very easily be overlooked. To overcome this difficulty a method was sought which would render the location and identity of the crown-gall organisms more easily discernible.

MATERIALS AND METHODS

The culture of *Ph. tumefaciens* used in this investigation was the progeny of a culture originally isolated from black raspberry and purified, after several platings, by single-cell isolation. The pathogenicity of this culture was repeatedly demonstrated on tomato, tobacco, and apple.

Readily discernible substances were sought which could be employed in conjunction with a suspension of the crown-gall bacteria at the time of inoculation to direct attention to the presence of the pathogene. It was hoped that such substances, when mixed with the living crown-gall bacteria, might be carried along with the bacterial mass. Then, by reason of their ready visibility, they might direct attention to the position of the crown-gall bacteria themselves. It was thought desirable that some of the substances should be living and some of them nonliving.

The substances employed included two living bacterial cultures and one inert substance. The cultures were *Ph. insidiosa* McCul. and *Ph. michiganensis* (Smith) Com. S. A. B., which are both Gram-positive and

both nonmotile. They were secured through the courtesy of F. R. Jones and H. L. Blood, respectively. Because of their characteristic reaction to Gram's stain, these organisms are very easily discernible within the tissue. A single rod of either species may be seen and identified as a bacterial organism inside the tissue with very considerable accuracy. The inert substance chosen was Burri's India ink. Different lots of these substances were employed alone or in different combinations as follows: (1) *Ph. tumefaciens,* (2) a mixture of *Ph. tumefaciens* and *Ph. insidiosa,* (3) a mixture of *Ph. tumefaciens* and *Ph. michiganensis,* and (4) a mixture of *Ph. tumefaciens* and Burri's India ink.

Control inoculations were made with the following materials: (1) Crown-gall bacteria, (2) a mixture of crown-gall bacteria and India ink, and (3) a mixture of crown-gall bacteria, India ink and *Ph. insidiosa.* In all cases galls developed at the end of the usual incubation period.

The movement of inert substances and a correlation of their reactions with those of the living bacteria under the conditions of puncture inoculation were considered. Such a study, it was thought, might facilitate the analysis of the factors responsible for the movement and the shape of the bacterial masses in the plant tissues. The materials chosen for this purpose were Burri's India ink and crown-gall bacteria which had been killed by heating to 75°–80° C. for 45 minutes. After heating the bacteria were plated on bile agar, but no growth appeared after an incubation period of 10 days. Smears of the heated bacteria were made on cover glasses and stained with safranin in order to determine their staining qualities. These appeared to be similar to those of the nonheated crown-gall bacteria. Two series of inoculations were made with these materials: One with India ink, and another with a mixture of the ink and the heated organisms.

All of the substances mentioned in this and the previous paragraphs were introduced by mean of needle punctures into the upper parts of young tomato plants which were four to five inches high. A loopful of the material was placed on the surface of the stem and a needle thrust made through the center of the drop and then into the stem.

Material for study was collected at the following intervals of time after the treatment of the stems: 2, 15, 30, 60, 180, and 240 minutes. The fixative most commonly employed was formal-acetic-alcohol, but Flemming's solutions were also tried. The use of these fixatives destroyed the mitochondria present and avoided the possible confusion of these bodies with the bacteria. The fixed pieces of stems, which were 1 to 1.5 cm. long, were dehydrated in alcohol, treated with chloroform, and infiltrated and embedded in paraffin in the usual manner. Sections were made at varying thicknesses between eight and twenty microns; Flemming's triple stain was ordinarily employed. This stain was varied when it was desired to demon-

strate Gram-positive organisms by introducing Lugol's iodine solution following the treatment with gentian violet.

EXPERIMENTAL RESULTS

The efficiency of the various substances which were mixed with the crown-gall organism with the hope that they would indicate its location considerably exceeded the expectation. The material treated with mixtures of the crown-gall organism and Gram-positive organisms showed the location of the different kinds of bacteria very conspicuously. The dark purple of the masses of Gram-positive bacteria was frequently located by a hasty survey of the section under low powers of the microscope. Under high magnification the Gram-positive rods were easily observed. About these dark purple rods or intermingled with them the red rods of the Gram-negative crown-gall bacteria were discerned. The India ink also served admirably to facilitate the location of the organisms introduced with it. The black particles of the ink appeared in sharp contrast against all the rest of the slide. The comparatively hyaline or red rods of the crown-gall organism could be observed among the carbon particles. When used alone the ink showed the probable path the bacteria would likely follow. It was found that the Gram-negative crown-gall organisms could be obscured by the Gram-positive organisms or the ink particles of the concentration of these substances was proportionately too great. A preponderance of the crown-gall organism over the other substances employed was consequently shown to be desirable. Within the length of time employed none of the ingredients of a mixed suspension was found to separate one from another. This technique served not only to facilitate the location of the "zoogloeal masses" of the bacteria, and to follow their movement, but also to find small groups of bacteria or even individual organisms. Without the aid of such easily discernible bodies many of the smaller groups of the crown-gall organisms would certainly have been overlooked.

The substances used, whether employed alone or in combinations, were found to be located in the cavity made by the needle, in the ruptured cells about the cavity, in the intercellular spaces of the different tissues, and occasionally in the ruptured vascular elements. Occasionally, even in material fixed only two minutes after puncturing, the entire cavity made by the needle was occupied by the bacterial mass. So far, no bacteria or carbon particles have been observed inside uninjured cells, although ruptured cells were often completely filled. The chief avenue of progress from the puncture into the surrounding tissue was observed to be the intercellular spaces. Although intercellular spaces, wherever they occurred, appeared to be channels for the movement of the various substances, those of the pith and subepidermal region were the ones commonly followed.

The movement in the intercellular spaces was found to be chiefly up and down from the puncture with comparatively little lateral movement. Occasionally the organisms or carbon particles were found to have penetrated into the vessels of the xylem for considerable distance. However, this took place only following ruptures of the vessels by the needle.

Within the intercellular spaces of sections cut longitudinally the various bacteria and India-ink particles were found in three different types of distribution: viz, (1) as long continuous or irregularly broken masses resembling "zoogloeal strands," which sometimes filled the entire intercellular space and sometimes occupied only one portion of it; (2) as clumps of various sizes usually in contact with the cell walls but occasionally appearing to be free inside the intercellular space; and (3) as individual particles distributed within the liquid which flooded the intercellular spaces at the time of wounding. No one of these three types of distribution has been found to occur over the range of material studied in much greater percentage than the others. The types of distribution in small clumps or as individual organisms were found to be much less conspicuous than that in "zoogloeal strands." It is likely that, without the aid of the agents used to facilitate their location, many of the less conspicuous groups and the individual bacteria would have been overlooked entirely.

The appearance of the "zoolgoeal strands" was very conspicuous particularly in cases which involved the mixture of the crown-gall organisms with Gram-positive bacteria or with India ink. More or less densely packed masses of bacteria were readily observed inside the puncture and "intruding" along the intercellular spaces from the puncture. Within the intercellular spaces the "zoogloeal strands" were found in several conditions. In some cases the entire space was occupied. However, the strands sometimes appeared only on one side as if they had progressed only in one angle of the intercellular space. Cross sections have repeatedly shown that this does occur. Also in large spaces the strands sometimes appeared as though they had occupied the entire space but had been shrunk, as if by plasmolysis. They occupied either a lateral or a central position and left a considerable part of the space unoccupied. In such cases, particularly when ink was employed, traces of the strand were found on all the walls. When the advancing tip of the strand was found it often was characteristically convex. This rounded tip was a very conspicuous feature of these strands. It appeared similar to those described by Nixon (4) and Hill (3). It occurred in the cases of dead bacteria and India ink just as it appeared with living organisms.

Clumps of bacteria were observed which were of different sizes, irregular in shape, and commonly appeared to be attached to the walls about the intercellular spaces. In such cases the middle portion of the inter-

cellular spaces usually seemed to be clear of any visible material. These phenomena were observed also when India ink and a mixture of India ink and crown-gall bacteria were employed.

Individual bacteria were found widely distributed in the intercellular spaces and gave an appearance very unlike that of "zoogloeal strands." Their distribution was followed from the puncture out towards the end of the section for several millimeters but no distinct slime, membrane, or a definite tip was seen. As a rule, they appeared widely scattered in the liquid which flooded the intercellular spaces. The denseness of their distribution diminished gradually with the distance from the puncture until finally none could be located.

Cross sections of the material for study showed the bacteria and the ink particles to be situated in three typical positions. (1) As individual particles or small clumps adhering on all sides of the intercellular space without any visible membrane enveloping them, (2) as small wedge-shaped masses occupying one or more corners of an intercellular space, and (3) as large masses occupying the entire intercellular space. In the last two cases the bacteria were seen as small dots embedded in slime. The blunt ends of the wedged-shaped masses, in some cases, appeared concave but in others they were convex.

A slimy matrix appeared to be associated with considerable concentrations of bacteria. This slime was frequently found in the needle cavity which was sometimes occupied completely. Commonly it appears to have shrunk considerably and to have assumed a reticulate character following fixation and staining. It has also been observed often in the intercellular spaces where it appeared to enclose the bacteria. The composition of this slime is uncertain. However, it appears likely that the slime produced by the crown-gall organisms in culture and the cell contents released from the injured cells at the time of puncturing might provide the necessary materials. The consistency seems such that it could flow readily through the intercellular spaces under the influence of the forces existing there. Not infrequently hyaline areas similar to those described by Nixon (4) were found around the bacteria in this matrix. These areas were interpreted to be vacant spaces caused by shrinking during fixation similar to those found about bacteria stained in the background of milk or broth.

The rate of migration of the bacteria in the tissues of the plant was considered. For this purpose material for microscopic study was taken at different intervals, as has been stated earlier, beginning with material fixed immediately after inoculation and extending through four hours. The distances traveled by the bacteria were measured from the margin of the puncture to the most remote bacteria observed in the intercellular spaces. In figure 1, where the results of these measurements are charted,

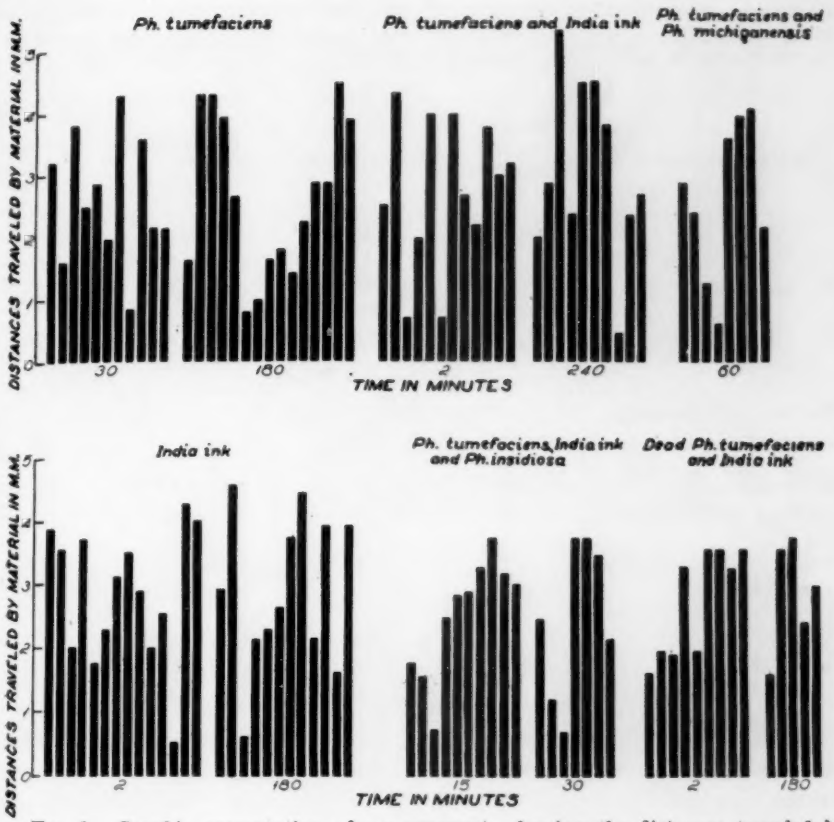

FIG. 1. Graphic presentation of measurements showing the distances traveled by various bacteria and inert materials, alone or in combination, in the intercellular spaces of tomato stems after stated intervals of time following their introduction through needle punctures.

it may be seen that there are no significant differences in the average distances traversed by the bacteria in the various time intervals. A summary of these measurements, showing the distances traveled by various bacteria and inert substances in the intercellular spaces after stated intervals of time, is given in table 1.

The trials performed with dead bacteria, India ink, or a mixture of both offer important evidence on the movement of the various substances in the intercellular spaces. These nonliving particles appeared in essentially the same distributions as the living bacteria. They formed similar strands and masses (Fig. 2, A–E) and were found also in small groups or as isolated particles. As in the cases with the living bacteria, there were no great differences in the average distances traversed during the various time intervals.

TABLE 1.—*Summary of measurements showing the distances traveled by bacteria and inert materials in the intercellular spaces of tomato stems after stated intervals of time following their introduction through needle punctures*

Materials introduced	Time between inoculation and fixation	Measurements	Range of distance moved	Average distance moved
	Minutes	No.	Mm.	Mm.
Ph. tumefaciens	30	11	0.83 to 2.24	2.61
	180	15	0.80 to 4.42	2.68
Ph. tumefaciens and India ink	2	12	0.70 to 3.90	2.76
	240	10	0.44 to 5.31	3.04
Ph. tumefaciens and Ph. michiganensis	60	8	0.61 to 4.07	2.59
Ph. tumefaciens, Ph. insidiosa, and India ink	15	10	1.72 to 3.71	2.54
	30	7	0.71 to 3.70	2.48
Dead Ph. tumefaciens and India ink	2	9	1.59 to 3.54	2.72
	180	5	1.59 to 3.54	2.86
India ink	2	14	0.53 to 4.24	2.92
	180	12	0.60 to 4.42	2.90

Experiments on the possible importance of capillarity were made in relation to the intercellular spaces. Small capillary tubes of glass were drawn to a size corresponding to intercellular spaces. The suspensions of bacteria and inert substances rose in them quite rapidly. The top of the column was always concave when the liquid rose under the influence of capillarity. However, when sufficient negative pressure was supplied, the top of the liquid was convex. The use of these tubes was open to objection because they provided a cylindrical column, while the intercellular air spaces usually possess several hollow wedges. Consequently, glass rods of a suitable size were drawn out and arranged in small bundles. The rise of liquid between them was studied. The meniscus was concave or convex, respectively, depending on conditions, just as it had been with the capillary tubes. However, because the rise was difficult to follow clearly when the rods were employed in bundles, they were laid as individuals upon long cover glasses so that they were in contact with the cover glasses for some distance. The cover glasses were stood on end, and a small drop of the liquid being studied was placed at the lower end. Its rise in the hollow wedges between the round rods and flat glass was followed with a low-power microscope by looking through the cover glass. As was to be ex-

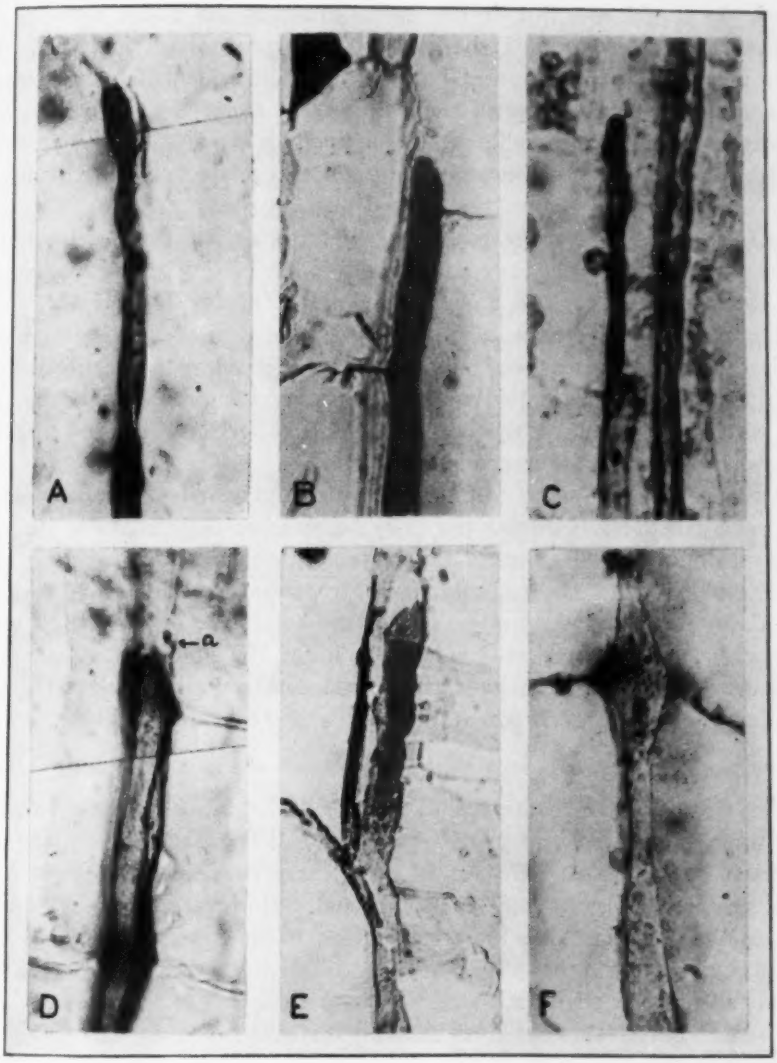

FIG. 2. Photomicrographs of Burri's India ink, crown-gall bacteria, and a mixture of both in the intercellular spaces of tomato stems. Magnification approximately × 1000.

A and B, Strands with convex ends of India ink.

C, D, and E, Strands with convex ends of a mixture of India ink and killed crown-gall bacteria. The bacteria and ink show more or less irregular distribution, sometimes appearing as clumps on the walls. In D particles of ink appear at *a* on the walls a short distance above the convex end of the strand. This suggests that the strand once stood at a higher position. Perhaps plasmolysis accounts for the withdrawal downward of this strand and the change from a concave to a convex shape at the tip. It probably accounts also for the clear spaces at the sides of the strand in both D and E.

F, Free crown-gall bacteria in the liquid released into the intercellular spaces by the puncture.

pected, the liquid rose first in the apical angle of the wedge between the glass rod and cover glass. The advancing tip representing the raised edge of the meniscus in the narowest part of the angle was ordinarily pointed, but sometimes when it stopped the liquid crept along the cover glass and appeared to form a more or less rounded tip. An attempt to provide small irregular spaces more like those in the plant was made by fusing grains of white sand, sifted to secure the desired size, to a cover glass. This was mounted under a microscope, cover glass up, and a small drop of the liquid placed at the edge. The movement was similar in the irregular space to that between the glass rod and cover glass. The rapidity of the movement in all these cases was influenced by the density of the bacterial suspension. When the bacterial slime was transferred without dilution directly from an agar slant the movement was much slower than in the case of a water suspension of the bacteria. This difference in speed appears to have been due to a difference in viscosity.

Although the subject of this study has been primarily the movement of the crown-gall bacteria during the early hours after their introduction into the stem of the tomato plant, a few sections were made through galls two to three weeks old. The technique employed was the same as in the case with the work on the early migration of the bacteria. The results secured have confirmed those reported earlier by Riker (5) and by Robinson and Walkden (7) that the organisms are located within the intercellular spaces and that they exert a cell-stimulating influence from that position.

DISCUSSION

The Gram-positive bacteria and Burri's India ink have been valuable aids, when mixed with cultures of the crown-gall organism, in locating this organism within the host tissue. In actual practice such mixtures were of much greater assistance than was expected. However, this technique is open to the objection that these substances, when mixed with the crown-gall organism, may provide a condition different from that in which the crown-gall organism is used alone. In both these cases the condition may also be different from that found during natural inoculation in the field. However, it appears unlikely that during the short time involved in these experiments, the Gram-positive bacteria and India ink have introduced any important error. On the other hand, they have increased so greatly the possibility of locating and observing the crown-gall bacteria that their use appears very advantageous.

The observations herein reported confirm those of Robinson and Walkden (7) and Hill (3) that the bacteria occur in masses embedded in slime which they call "zoogloeae" and "zoogloeal strands." However, this is not the only form in which the bacteria appear in the early stages after in-

oculation. Small clumps, as well as individual bacteria, have been found commonly to occupy intercellular spaces several millimeters long. These findings emphasize Riker's (5) earlier report of the importance of the liquid which flooded the intercellular spaces following the rupture of the cells in the path of the inoculating needle. In this connection it is noteworthy that this liquid contains the entire cell contents and not merely the cell sap. Consequently, a considerable part of it is not subject to reabsorption into the surrounding tissue as suggested by Hill (3).

The conception that the movement of the organisms through the host tissue is due to growth has not found support in these studies. Four lines of evidence oppose this idea. (a) No significant progressive movement has been found for the different time intervals. (b) Such inert substances as dead bacteria and India-ink particles have shown a similar movement to corresponding distances. (c) The lag period, found by Wright, Hendrickson, and Riker (8) following transfers of the crown-gall organism, indicates that vigorous growth does not occur until considerably later than the times considered in this work. (d) The earlier report by Riker (5) that the galls developed within the general region involved by flooded intercellular spaces after puncturing has been repeated and confirmed many times. If the crown-gall organism were capable of active migration by growth, the crown galls which follow puncture inoculations would probably not be so definitely limited.

The mechanism of the movement of the bacteria is not definitely understood. Since it appears that growth has little or nothing to do with this movement within a few hours after treatment, it seems that various physical forces, including capillarity, convection currents, and negative pressure, may be responsible. Under the conditions of these experiments, motility by its flagella of the crown-gall organism seems to have little to do with the progress of the organism in the tissue except, perhaps, in the cases of isolated bacteria. The rapidity of the movement first suggests that either negative pressure or capillarity is primarily responsible for this movement. Under the influence of negative pressure a convex tip would be expected on "zoogloeal strands." It is possible that, after rising by capillarity, the concave tip of a "zoogloeal strand" may become somewhat "plasmolized" during the process of preparing the material for examination and change into a convex form. It is well known that when cell contents become plasmolized they withdraw from the corners of the cell walls and commonly appear comparatively convex, rather than angular according to the form of the wall. That such a thing does at times take place in the case of bacterial masses in the intercellular spaces is suggested by a number of tips, one of which is shown in figure 2, D. Particles of ink may be seen adher-

ing to the wall beyond the tip of the strand. In this case it is probable that plasmolysis has turned a concave tip into one that is convex. It seems unlikely that such details could be observed when the crown-gall bacteria are employed without the addition of the ink. In this same figure there also is evidence at the side of the strands that shrinkage has occurred.

The collection on the intercellular walls of thin layers of bacteria and clumps of various sizes, leaving the middle portion of the intercellular space free, may also be influenced by physical forces, including shrinkage. It is considered that originally these bacteria were more or less evenly distributed in the liquid but under the influence of different reagents it shrunk, accumulated upon the cell walls, and left the middle of the intercellular space apparently free.

In consideration of the available evidence it appears that, instead of growth, various physical factors, including perhaps water content of the host plant, release of cell contents by the needle puncture, bleeding of the host plant, capillarity, diffusion and negative pressure, may influence the movement of the crown-gall bacteria and the manner of their distribution within a few hours after inoculation under the conditions studied. The form of these strands as they appear in histological preparations, however, may depend upon the shape of the intercellular space and the degree of shrinkage. Later on, growth and metabolism of the organism and the various physiological responses of the host plant, together with these physical factors, may be influential in the progress of the bacteria through the tissue. In this connection osmotic pressure must certainly be taken into account. A consideration of this phase of the subject is beyond the scope of this paper.

SUMMARY

1. Finding the crown-gall bacteria in the stems of tomato plants a few hours after their introduction through needle punctures was facilitated by mixing Gram-positive bacteria or Burri's India ink with the inoculum.

2. The bacteria were found in the cavity made by the needle, in ruptured cells, and in the intercellular spaces. They occurred in the stained preparations as strands which were sometimes continuous and which had convex tips, as clumps of a number of individuals, and as individual cells. All three types of distribution were observed in the same slide.

3. The distances traveled by the bacteria within the first four hours after introduction were quite variable. No significant progressive movement attributable to growth was observed.

4. Dead crown-gall bacteria and particles of India ink showed the same types of distribution and moved approximately the same distances as the living crown-gall bacteria.

5. The mechanism by which the bacteria and inert substances employed in these experiments are moved through the intercellular spaces is not definitely understood. Several lines of evidence oppose the concept that the movement studied was the result of growth. Various physical forces, particularly capillarity and negative pressure, seem important. The physiological disturbances incident to the inoculations also deserve consideration.

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SOME POSSIBLE CAUSES OF STREAK IN TOMATOES[1]

W. D. Valleau and E. M. Johnson

In studying the problem of the virus diseases of tobacco, as they occur in Kentucky, one is impressed by the increasing number of what appear to be distinct strains of several groups of viruses. The diseases produced by the different viruses often can be distinguished only by a comparative study under controlled conditions. Several of the tobacco viruses under study are likewise found in tomatoes, causing a disease which might logically be called tomato mosaic. Therefore the terms tobacco mosaic and tomato mosaic have but little meaning to a student of the virus diseases of these crops and merely lead to confusion when used in this way in the literature. This statement may need some qualification with respect to true tobacco mosaic, typical cases of which are recognized by most pathologists, but even true tobacco mosaic may be caused by several distinct virus strains. It now appears to be agreed that tomato streak is caused by a virus or a mixture of viruses but there seems to be doubt as to the exact identity of the viruses concerned. One of them is the virus present in the so-called healthy potatoes. The identity of the others, if more than one is concerned, is certainly a matter of conjecture. As the writers have a considerable number of distinct virus diseases under study, practically all of which were obtained originally from naturally infected tobacco, an attempt was made to clarify some of the confusion which appears to exist as to the cause of streak in tomatoes.

LITERATURE REVIEW

As the literature on streak has been reviewed recently (1, 9) it is hardly necessary to mention more than a few recent papers. Gardner and Kendrick (2), in discussing mosaic of tomatoes, pictured streak as one of its symptoms, thus recognizing the virus nature of the disease. Vanterpool (9) observed the significant fact that if streak material is dried from 3 to 9 months and then inoculated into tomato plants, it no longer produces streak but only a severe case of mosaic. He thus demonstrated that a strain of true tobacco mosaic was present. Acting on the suggestion obtained from a report by Johnson (3), he inoculated tomato plants with tomato mosaic and an extract from mosaic potatoes and obtained what he considered to be typical streak.

Berkeley (1), while admitting the virus nature of streak, states that "the experimental evidence reported here does not agree with the theory

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

of a combination of tomato and potato mosaic viruses as the true cause of streak.'' He claimed that juice from healthy potato plants produced definite streak in tomatoes, as well as that from mosaic potato plants, and from potatoes with streak. Vanterpool had been unable to produce streak in tomatoes by inoculating with potato streak material. Berkeley also noticed apparent differences in rate and ease of spread of streak in various places and likewise differences in symptoms, which appeared to be of significance. Smith (5), in studying the transmission of a potato-mosaic virus to tomatoes, found that a mixture of the potato virus with a yellow mosaic will cause a severe disease which has all of the characteristics of streak. The mixed-virus theory of the disease is also confirmed by Stover (6), who showed that mosaic tomato plants developed streak when inoculated with the juice of potatoes affected with mild mosaic, rugose mosaic, leaf roll, spindle tuber, or apparently healthy potato. While there appears to be a multiple cause of streak in these experiments, all may be interpreted as confirming the tomato mosaic plus healthy-potato virus theory of the disease, as it is entirely probable that the various potato plants used all carried the healthy-potato virus in addition to some other virus, the latter either alone or in conjunction with the former resulting in the symptoms described as rugose mosaic, leaf roll, etc.

FIVE GROUPS OF VIRUSES AFFECTING BOTH TOBACCO AND TOMATO

For the past several years the writers have been studying the virus diseases of tobacco as they occur on the Experiment Station Farm at Lexington and vicinity and have obtained a few tobacco viruses from other States (7). The Experiment Station Farm is particularly well supplied with virus diseases, probably because potatoes, tobacco, and tomatoes have been grown more or less regularly for many years and because perennial weed hosts are abundant. Cultures of all the virus diseases which in any way appear to differ have been collected and then passed through a succession of Turkish tobacco plants in the greenhouse and the symptoms compared. In this way it has been possible to separate virus diseases which superficially appear alike and to group them on the basis of similarities of symptoms. At least five groups of viruses that affect both tobacco and tomatoes have been recognized. These are the true tobacco-mosaic group, characterized by the well-known symptoms of tobacco mosaic, having the unique ability of living for years in dried tobacco material, and apparently affecting only members of the Solanaceae; the etch group, characterized by a fine necrotic etching on the rubbed leaves and on one or more leaves which first develop after inoculating Turkish tobacco plants; the viruses of the cucumber-mosaic group, best characterized by their ability to cause mosaic in

cucumbers (tobacco ring spot will also do this but should not be confused with the cucumber mosaics); the veinbanding virus, causing a disease in tobacco fields where potatoes have formerly been grown but causing very faint symptoms in tomato; and the virus obtained from so-called healthy potato, which will be designated as healthy-potato or the potato virus in this paper. The disease caused by the healthy-potato virus used in these experiments is of the type designated by James Johnson (4) as "ringspot."

The studies to be reported have been conducted with certain of the above-mentioned virus strains which were thought to be pure or to contain but a single virus strain. It must be stated that there is no absolute certainty on this point, but it is probable that if mixtures occurred they were between strains of the same type of virus so much alike as not to affect the results. Small numbers of plants have been used throughout the tests, a fact which might bring their accuracy into question, but, as the methods of handling the virus diseases have been refined over a period of years to a point where accidental infections rarely occur and as transfers have been made back to tobacco plants in all cases, we have confidence in the results. The method of inoculation used is that of crushing the viruliferous material in a sterile culture dish with a stick bound on the end with a piece of cheesecloth and then sterilized. A piece of waxed paper is laid on the left hand, the leaf to be inoculated is supported on this paper, and the padded stick used to rub the inoculum on the leaf. Although many virus strains are kept in one house, accidental infections are so rare as to be nearly negligible.

The tomato plants were grown in half gallon or two gallon jars of forest soil and were kept in a vigorous growing condition by frequent applications of nitrogen salts.

The healthy-potato virus used in two experiments was obtained from a very mildly mottled tomato plant growing in the Horticultural Greenhouse not far from Irish Cobbler potatoes. This virus strain had been studied for over a year in comparison with the virus obtained directly from these Cobblers, and no differences could be noted in Turkish tobacco plants infected with them. In a third experiment the virus used was obtained originally from an apparently healthy Irish Cobbler plant.

TOBACCO MOSAIC VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with the potato virus, with three distinct strains of true tobacco mosaic, and with these in combination with the potato virus. Mild mosaic alone caused very mild symptoms, the plants appearing nearly healthy. Severe mosaic caused leaf distortion, blistering, mottling and no necrosis. The healthy potato virus alone caused faint mot-

tling but no distortion or necrosis. In combination with healthy-potato virus mild mosaic resulted in a mild form of stem streak with leaf necrosis, faint mottling, and slight leaf distortion. The potato virus in combination with severe mosaic resulted in streak of a more severe form. Stem and petiole streaks were numerous and prominent, leaf necrosis was severe and the leaves were distinctly mottled and distorted. Yellow tobacco mosaic, a disease very similar to severe tobacco mosaic except that yellow blotches are produced in the leaves, in combination with the potato virus caused streak of about the same degree of severity as that resulting from severe tobacco mosaic in combination with the potato virus. In tomato the yellow blotches are produced as in tobacco.

Inoculations were made from the affected tomato plants to Turkish tobacco. The combination viruses in each case produced symptoms typical for the tobacco virus concerned except that small necrotic rings typical of the potato virus usually developed on rubbed leaves and small necrotic spots developed on the later mottled leaves. The single viruses in each case gave the expected results.

CUCUMBER MOSAIC VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with three strains of cucumber-mosaic (Types 1, 2, and 3) which produced distinct symptoms on both tobacco and cucumbers, and with these in combination with the potato virus. Type 1, which represents the cucumber mosaic most commonly found in cucumbers and tobacco in Kentucky, caused narrowing of tomato leaves but no necrosis except on rubbed leaves. Type 2 caused no leaf distortion or necrosis but the leaves produced subsequent to inoculation were mottled with ring-like patterns. Cucumber mosaic type 3 causes severe distortion in tobacco and results in fern-leaf and slight leaf necrosis of unrubbed leaves in tomato. While the cucumber mosaics inoculated in combination with the potato virus did not produce streak so consistently as the true tobacco mosaics yet streak was produced with each of the three types. Failure of the cucumber mosaics to produce streak in certain instances appeared to be due to difficulty in transferring these viruses to tomato. Cucumber mosaic type 3 plus the potato virus produced more severe streak symptoms than either of the other two types and in one experiment this virus combination produced more severe symptoms than any other combination tested. In one instance the fruit on a streak plant was spotted with small brown raised lesions on the surface of the fruit. This was the only case observed where the fruits showed symptoms except those on plants affected with ring mosaic. The absence of fruit symptoms is probably of little significance as the plants could not be kept in as vigorous a state of growth in jars as in

benches of soil. Inoculations from the affected tomato plants to tobacco indicated that accidental infections with the true tobacco mosaic viruses had not occurred in the case of streaked tomato plants, although one tomato plant inoculated with cucumber mosaic type 3 alone was found to have contracted tobacco mosaic toward the end of the experiment. Tobacco plants affected with the mixed viruses, cucumber mosaic and healthy potato, developed symptoms typical of the cucumber mosaic concerned but with some leaf necrosis.

ETCH VIRUSES PLUS HEALTHY-POTATO VIRUS CAUSE STREAK

Tomato plants were inoculated with three strains of the etch virus, etch, etch +, and severe etch, each capable of causing a disease of a different degree of severity in tobacco. These were also used in combination with the healthy-potato virus. The etch viruses alone cause narrowing, marked distortion and mottling of tomato leaves. The diseases produced could readily be mistaken for those produced by the more severe strains of tobacco mosaic. Plants affected with severe etch were always more stunted than other plants in the experiments. Each of the etch viruses in combination with the healthy-potato virus caused stem and petiole streak and extensive leaf necrosis. Etch +, which produces more marked symptoms in tobacco than etch, caused more severe streak on tomato when combined with the potato virus than etch. Transfers from the affected tomato plants to tobacco gave the expected results indicating that accidental infection with tobacco mosaic had not occurred. The potato virus caused slightly more pronounced necrosis in tobacco when combined with an etch virus than the etch virus alone.

RING MOSAIC VIRUS USED ALONE PRODUCES STREAK[2]

The ring mosaic virus, so named because it produces symptoms somewhat similar to tobacco mosaics but with a distinct tendency for the mottling to be in the form of rings, appears to be of the same nature as the true tobacco mosaics in that it withstands drying. This virus in combination with the healthy-potato virus causes a severe stem and petiole streak and leaf necrosis. When used alone it also causes stem and petiole streak and leaf necrosis, but perhaps slightly less severe than is caused by the mixture. The virus has produced streak in tomatoes when transferred either from green tobacco plants or from dried tobacco. As the healthy potato virus does not appear to be able to withstand drying the possibility of a mixture with it seems to be eliminated. Fruits produced on tomato plants affected with ring mosaic often develop depressed chlorotic rings of varying size. The rings sometimes become necrotic soon after they develop resulting in

[2] This virus disease of tobacco was earlier designated C.T. (6).

misshapen distorted fruits. Transfers from the affected tomato plants to tobacco gave the expected results, with slightly increased leaf necrosis in the case of the mixture.

VIRUS COMBINATIONS WHICH DID NOT PRODUCE STREAK

All of the possible combinations of the different strains of viruses under study were not tested for their effect on tomatoes. The possible combinations of virus groups, except vein banding, were made but none in which the healthy-potato virus was not present produced streak or leaf necrosis. The combinations tested were: Cucumber mosaic type 3 plus severe tobacco mosaic, cucumber mosaic type 2 plus etch +, and etch + plus mild tobacco mosaic. A combination of veinbanding with the healthy-potato virus failed to produce streak in tomatoes although it sometimes results in slight-leaf necrosis. This combination frequently causes streak in seedling potatoes and the veinbanding virus alone when passed to Cobbler potatoes frequently causes leaf necrosis and streak, although the latter symptoms usually disappear if the plants make further growth.

DISCUSSION

The results have shown clearly that streak of tomatoes may be caused by each of the virus strains tested except veinbanding, when used in combination with the virus from apparently healthy potatoes. One virus of the true tobacco-mosaic group, designated ring mosaic, was found to produce streak when used alone. It produces depressed, somewhat chlorotic rings sometimes half an inch in diameter on the surface of the tomato fruits, and can thus be easily identified.[3] Ten cases of tomato streak were produced by inoculating 10 tomato plants with dried tissue of plants infected with this virus, thus tending to eliminate the possibility of its being a mixture with other than true tobacco mosaics.

While all possible combinations of the virus strains were not tested, the possible group combinations, except veinbanding, were tried with the result that no combination in which the potato virus was not present produced streak.

It is thus seen that streak is a symptom with little or no etiologic significance except that it indicates a virus disease of tomatoes due, in certain instances, to a mixture of viruses, one of which is likely to be the virus com-

[3] This disease of tomatoes is apparently identical with one of the streak diseases reported at the Des Moines meeting by S. P. Doolittle and H. L. Blood (Abstract in Phytopath. 20, 1930) in which similar rings on the fruit were illustrated. Doctors Max W. Gardner and J. B. Kendrick informed the writers that similarly marked fruit is often seen in shipments of tomatoes from California. Inoculations from what may have been one of these fruits was reported on in Brewer, Kendrick and Gardner. Effect of mosaic on carbohydrate content of the tomato plant. Phytopathology 16: 843–851. 1926.

monly found in apparently healthy potatoes. This raises the question as to the cause of streak in epiphytotics. That it will be found to be caused by different viruses or mixtures of viruses is to be expected from the published reports of other investigators. Berkeley claimed to be able to produce streak with the juice of healthy, mosaic, and streak potato plants. The cucumber-mosaic viruses and the etch viruses are each capable of infecting potatoes and may have been concerned in Berkeley's experiments, or it is not impossible that viruses other than those so far recognized may have been present in his potatoes and, together with the healthy-potato virus, may have caused streak when transferred to tomatoes. Considering the large number of virus diseases of potatoes and the almost universal presence of at least one virus in all apparently healthy potatoes, it would not be surprising if, even in seemingly healthy potatoes of certain varieties, a virus combination were found, capable of causing streak in tomatoes. In the present studies we have seen no indication that the virus commonly present in healthy potatoes, when used alone, can cause other than a mild mottling in tomatoes.

In epiphytotics of streak in tomatoes, it is desirable that the particular viruses concerned be determined, as this knowledge may assist in determining the source of the trouble. If only tomatoes are used as a host for a study of the virus symptoms, determinations are nearly impossible. Turkish tobacco has proved valuable for this purpose because the plants are relatively small and occupy but little bench space and because the symptoms of the various virus groups are distinct enough to make determinations comparatively easy. If transfers are made from streaked tomato plants to Turkish tobacco by the rubbing method, both viruses concerned are likely to be transmitted to the tobacco. Usually the healthy-potato virus will manifest itself by the presence of small necrotic rings on the rubbed leaves and by a slightly increased amount of necrosis in later leaves, if it is in combination with another virus. The other virus will usually produce nearly its typical symptoms, which may be recognized. For those not familiar with the symptoms of the virus groups here discussed, further tests may be necessary.[4] If the streak material is dried and inoculations of tomato or tobacco plants produce a mosaic, it is almost certain to be one of the strains of true tobacco mosaic. For comparison, true tobacco mosaic may be readily obtained by moistening tobacco of nearly any of the common brands of cigarettes or smoking tobaccos and rubbing this decoction on healthy tobacco leaves. If the virus from streak plants does not withstand drying, it may be the virus of one of the cucumber mosaics. This may be deter-

[4] A more extensive description of the virus diseases concerned in the present studies will be found in Kentucky Exp. Sta. Bul. 306.

mined by inoculating cucumbers. If mosaic develops, it may be considered proof that one of the strains of cucumber-mosaic virus is involved, as at present there is only one other known cause of mosaic in cucumbers, namely, tobacco ringspot, and this will not transfer readily to tomatoes. If one of the etch group of viruses is concerned, the fact will readily be recognized if young, vigorously growing Turkish tobacco plants are inoculated with the streak tomato juice. The fine necrotic etchings which appear on the upper surface of one or more of the new leaves of Turkish tobacco following inoculation are very characteristic. In view of the fact that members of these virus groups are capable of producing streak on tomatoes when inoculated together with the potato virus, it would not be surprising if other viruses also may be found to cause streak of tomatoes when mixed with the healthy-potato virus.

A determination of the viruses concerned in streak may suggest its origin and consequently may assist in control. If the healthy-potato virus is one of the complex, it immediately suggests potatoes as at least one source. If the true tobacco mosaic is one of the complex, in a region where tobacco is not commonly grown, personally used tobacco is suggested as a possible source of this virus, as it is readily transmitted to tomatoes if the hands become contaminated by handling viruliferous smoking or chewing tobacco; or they may become contaminated by handling unheated tobacco stems, commonly used for fumigating greenhouses.

The cucumber-mosaic viruses and the etch viruses may originally have come from potatoes, as both appear to be transmissible to and from potatoes by insects or they may come from weeds (8). It has been observed that one of the etch viruses will spread very rapidly through a tomato planting in the field if a source of inoculum is present.

The seeming failure of *Myzus persicae* to transmit the healthy-potato virus[5] suggests that its presence in tomatoes in the greenhouse may result from handling potatoes or potato plants carrying the virus and subsequently handling tomato plants. It thus appears that mechanical as well as insect transmission must be given consideration in attempting to explain original infections of tomatoes with streak and its subsequent spread.

CONCLUSIONS

1. A study has been made of the possibility of causing streak in tomatoes with various tobacco viruses, both in combination with the so-called

[5] This is based on the belief that the ringspot of tobacco, as reported by K. M. Smith (Ann. Appl. Biol. XVI, No. 3, 1929) is caused by a mixture of the healthy-potato virus and his insect-transmitted virus, which produces a disease in tobacco which we have previously named veinborder or veinbanding. Smith has assumed that apparently healthy potato plants are virus-free because of his failure, following inoculation to tobacco, to obtain recognizable symptoms.

healthy-potato virus and in other combinations in which the healthy-potato virus was not included.

2. Streak has been produced by an unmixed virus apparently belonging to the true tobacco-mosaic-virus group; by three other strains of the true tobacco-mosaic viruses plus the healthy-potato virus; and by three strains of the cucumber-mosaic viruses plus the healthy-potato virus; and by three strains of the etch virus plus the healthy-potato virus.

3. Streak was not produced by a mixture of one of the etch viruses and a virus of true tobacco mosaic, by the cucumber-mosaic virus plus true tobacco-mosaic, by cucumber-mosaic virus plus the etch + virus, or by the healthy-potato virus plus veinbanding.

4. The virus strains within a given virus group differ primarily in severity of symptoms produced on a given host. In the three virus groups under study, the one in each group producing the most severe symptoms on tobacco resulted in the most severe streak of tomatoes when combined with the potato virus.

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THE LONGEVITY OF PHYLLOSTICTA SOLITARIA E. AND E. ON APPLE SEEDLINGS HELD IN COLD STORAGE

J. A. McCLINTOCK

Gardner and Jackson[1] have pointed out the relationship of blotched apple seedlings to the spread of *Phyllosticta solitaria* in Indiana, and observations in Tennessee since 1923 fully substantiate their findings.

Since most of the blotch cankers on apple seedlings are above the crown, it is to be expected that there will be less spread of the causal fungus from infected seedlings to scions in nurseries where most of the propagation is done by whip grafting. Nearly all of the seedling top is discarded in making piece-root grafts, yet the danger from blotch-fungus-infected seedlings is not entirely eliminated, as is shown by cases of well-developed blotch cankers on the trunks of grafted apple trees, both below and above the union.

Examination of apple seedlings shipped into Tennessee since 1923 has shown the presence of varying amounts of blotch-fungus infection from year to year. Since blotch-infected seedlings are found continually in shipments, nurserymen should cull, on arrival from seedling growers or dealers, all trees which show such cankers.

APPLE BLOTCH ON SEEDLINGS FROM COLD STORAGE

The extent to which fruit-tree seedlings are held in a dormant state, from one year to another, in cold storage is not definitely known. However, two cases are known where, in years of heavy seedling production, a Tennessee nursery held surplus lots of 50,000 and 60,000 apple seedlings in cold storage throughout an entire growing season and then used them the following year.

The apple seedlings from which the following data were obtained were grown in midwestern seedling nurseries in 1927 and shipped to the purchaser in the winter of 1927–28. They were held in paper-lined boxes in nonheated rooms until May 1, 1928. Then 60,000, in their original packing boxes, were placed in a commercial cold storage. They were held at temperatures ranging from 34° to 36° F. No moisture was added during the storage period. On February 8, 1929, the boxes of seedlings were removed after being in cold storage for nine months. They were taken to the nursery to be used for propagating purposes.

A few hundred of these stored seedlings were obtained for tests at the Tennessee Agricultural Experiment Station in comparison with seedlings

[1] Gardner, Max W., and H. S. Jackson. New aspects of apple blotch control. Phytopath. 13: 44. 1923.

grown in 1928. While examining these cold-storage seedlings, preparatory to lining out in test rows at the University farm, the writer observed typical blotch cankers. This test lot was then carefully sorted and the seedlings having the largest number of cankers per tree were selecteed. The cankers appeared similar to those found from year to year on seedlings raised in midwestern nurseries. Careful examination of each canker showed no evidence of enlargement of the original cankers during the cold-storage period of nine months.

THE DEVELOPMENT OF BLOTCH-FUNGUS CANKERS ON POTTED SEEDLINGS

According to Roberts,[2] the blotch-fungus in the older portion of a canker dies. As these stored seedlings showed no evidence of canker growth for more than a year, it seemed desirable to determine whether the cold-storage period to which the seedlings had been subjected had eliminated the danger of spreading *Phyllosticta solitaria* from the cankers formed during 1927. Seedlings from the most heavily infected lot were root pruned, and set in large pots of soil on March 8, 1929. Among these potted seedlings were planted apple seeds freshly removed from healthy Yates apple fruits. The pots were labeled and set on a greenhouse bench for observation. Buds on some of the stored seedlings began to show signs of growth within three days and, in most cases, the seedlings developed normally. Some seedlings in each pot failed to start and within a few weeks died. Somewhat later additional seedlings appeared in the pots from the Yates seed planted March 8, 1929.

As the season advanced, temperatures within the greenhouse became too high for the best growth of the seedlings, so the pots were moved to a plat outside the greenhouse but near enough to it to be watered. As seen in figure 1, these potted seedlings made fair growth, considering the restricted soil area to which they were confined.

As the growing reason advanced, it was obvious that the blotch fungus was spreading from the old cankers into the surrounding stem tissues of the potted seedlings. Eventually pycnidia appeared on the surface of the new canker tissues formed about the old cankers. This indicated that *Phyllosticta solitaria* in cankers formed on the seedlings in the midwestern nurseries in the summer of 1927 had remained alive but dormant until conditions became again favorable for its development in the spring of 1929. Following the development of new canker growth on the stored seedlings, as seen in figure 2, *1* typical blotch-fungus infections began to appear on the leaves, which were chiefly on the new growth above the cankers. In contrast the leaves on the small Yates seedlings were heavily

[2] Roberts, John W. Apple blotch and its control. U. S. Dept. Agr. Bul. 534. 1917.

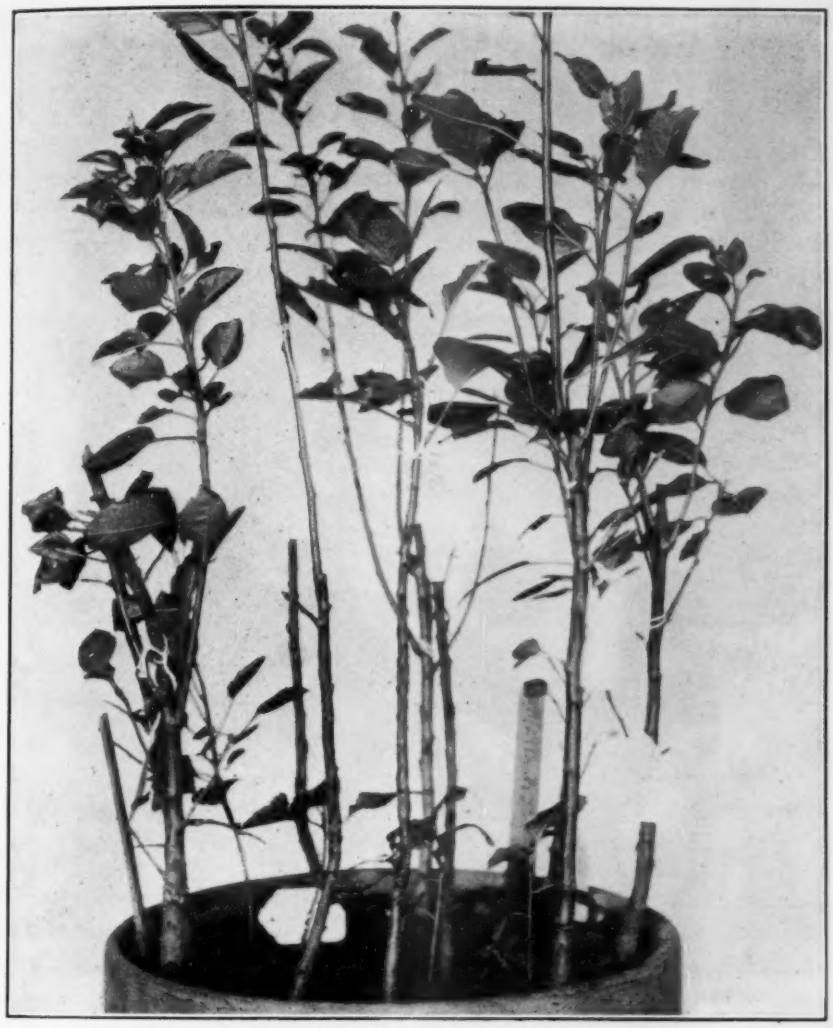

FIG. 1. Cold-storage seedlings, showing most of the 1929 twig and leaf growth above the 1927 trunk cankers. The small seedlings are from Yates seed planted in the pot.

infected, as seen in figure 3, *1*. This difference was chiefly due to the fact that the Yates seedlings being shorter had more leaves below the cankers where spores readily fell upon them. The majority of the leaf spots developed single pycnidia, the characteristic form which the specific name of the fungus is derived.

On August 24 typical petiole cankers (Fig. 2, *2*) were found on several leaves on the small Yates seedlings. These petiole cankers contained

numerous pycnidia. In a single case *Phyllosticta solitaria* had progressed from one of these petiole infections and formed a small but typical canker on the trunk of a Yates seedling. This canker could not be photographed as it grew in the pot, and it was not desirable to sacrifice the seedling for a photographic record at that time.

As there was no other near-by source from which the leaf spot and petiole infections could have developed, their presence on the Yates seedlings indicated that infection came directly from the renewed growth of the blotch fungus from the old cankers on the stored seedlings.

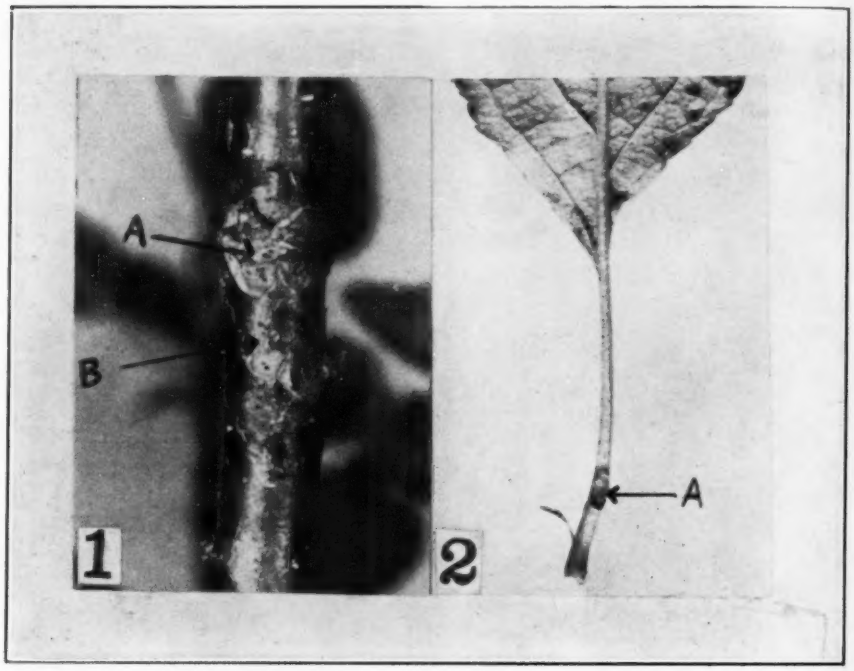

FIG. 2. *1.* An enlargement of a potted cold-storage seedling, showing the 1927 canker at A and at B the new canker tissues produced in 1929 by an outgrowth of the blotch fungus from the 1927 canker.

2. Leaf from Yates seedling growing in pot with the cold-storage seedlings. Note the typical petiole infection of the blotch fungus at A.

Of a total of 83 blotch-fungus cankers on the potted seedlings, six showed no signs of new growth and appeared to be dead, while 77 showed typical new growth, as seen in figure 3, *1*. In nine cases *Phyllosticta solitaria* had grown from old cankers directly into new growth produced by buds adjoining the old cankers, thus enlarging the old cankers on both the 1927 and the 1929 growth. These facts indicate that *P. solitaria* will live for nine months on seedlings held at temperatures of 34° to 36° F.

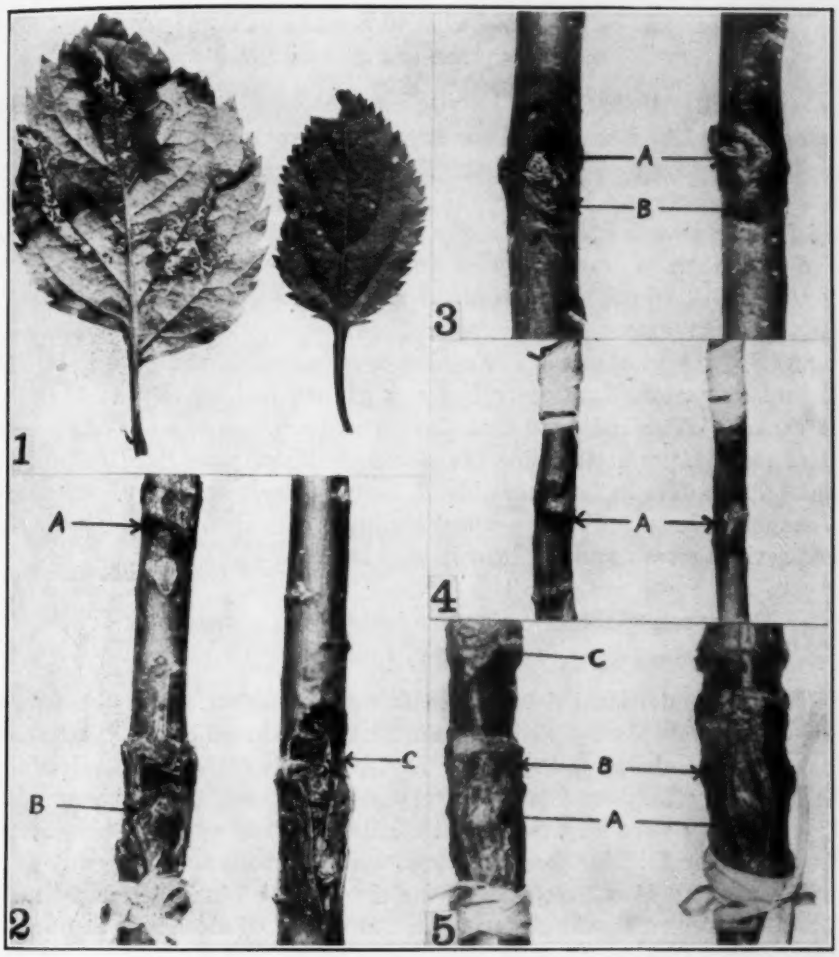

FIG. 3. *1.* Leaves from Yates seedlings growing in pots. Note the circular spots on the upper surfaces of the leaves, with single pycnidia typical of blotch leaf infection.

2. Cold-storage seedlings collected in a commercial nursery September 7, 1929. On the seedling to the left the 1927 blotch canker, with new 1929 growth at A, is well above the inserted bud B and would be removed in cutting the seedling top back to the bud. On the seedling to the right the new blotch canker growth at C adjoins the inserted bud so closely that it could not all be removed in topping.

3. Lined-out apple seedlings taken from the test plat August 26, 1929. Note the 1927 cankers at A and the new 1929 canker growth at B produced by the growth of the fungus from the old cankers.

4. Two cold-storage seedlings collected in a commercial nursery on September 7, showing the new 1929 blotch canker growth at A. These cankers below the buds, which are enclosed in the raffia wraps, would not be removed in topping the seedling stocks.

5. Cold-storage seedlings collected in a commercial nursery September 7, 1929. The vertical and horizontal cuts on the seedling stocks are directly into blotch cankers. The inserted buds at A are almost surrounded by the new 1929 blotch-canker tissue B, which developed from 1927 cankers like C.

DEVELOPMENT OF PHYLLOSTICTA SOLITARIA IN CANKERS ON STORED SEEDLINGS LINED OUT IN THE FIELD

On April 8, 1929, the less heavily infected seedlings from cold storage were lined out in a section of nursery row for comparison with nonstored seedlings as to growth and vigor. While the percentage of loss through subsequent death was higher in the stored seedlings, those that lived made satisfactory growth and were ready to bud as soon as nonstored seedlings of the same grade growing under similar conditions.

On August 26 the lined-out blotched seedlings were examined to determine to what extent the fungus had developed under field conditions. Figure 3, *3* is typical of the new canker development in these seedlings. In all cankers examined, more or less new growth had developed about the old cankers. This indicated that *Phyllosticta solitaria* was alive in nearly all of the cankers at the time the stored seedlings were lined out in the field. These data indicate that blotch-fungus-infected stored seedlings are as dangerous in spreading the causal fungus as are infected seedlings grown the previous season and not held in cold storage.

BLOTCH CANKERS ON STORED SEEDLINGS LINED OUT IN A COMMERCIAL NURSERY

To obtain additional data on the extent to which *Phyllostica solitaria* lives over on cold-storage seedlings, the nursery from which the stored seedlings came was visited on September 7. This nursery, about 200 miles from the University farm, consists of several separated farms. The farm on which these seedlings were set was at least a mile from any apple nursery stock. The particular field had been in a rotation of general farm crops, and no apple trees had been grown there for six years. With such isolation it would be expected that the existing blotch-fungus infection had been introduced on infected seedlings.

As is the practice in Tennessee nurseries, the majority of the seedlings removed from cold storage on February 8 had been used for whip grafts. These grafts, which included a number of commercial varieties, had been lined out according to nursery practice and were making satisfactory growth. As the graft unions in all cases were well below the ground, the seedling stocks could not be examined for blotch cankers. A careful examination of the old and new scion growth aboveground showed no evidence of blotch infection on thousands of whip grafts, many of which are susceptible varieties.

Adjoining the block of whip grafts was a block of 5,000 of the seedlings from cold storage which had been lined out in April, 1929. These seedlings

had made good growth and had been budded during the latter part of July and early August. In nearly all cases the buds were still covered with raffia wraps, but active blotch cankers were readily found on the seedling stocks, as seen in figure 3, *4*. Such cankers on the seedlings lower than the points where buds were inserted might easily be overlooked by budders and probably would not be detected in subsequent field operations. Spores liberated from pycnidia in these cankers would be a ready inoculum for new bud growth the following season. On further examination seedlings like those of figure 3, *2*, were found with active blotch cankers both above and adjoining the inserted buds. The cankers some distance above the buds would probably not be a source of danger because cutting back the tops to the buds the following spring would remove them before new bud growth started. In the case of cankers adjoining the inserted buds, however, the danger of infection would be much greater, since infected tissues would not be entirely removed in cutting the tops back to the buds in the spring. The fungus in that part of the canker left on the seedling stock could attack the bud either by direct mycelial growth as the canker enlarged or by spores from pycnidia attacking the new growth from the inserted bud.

It is not a general practice to give apple nursery stock the frequent and thorough Bordeaux sprays which are effective in controlling blotch of bearing orchard trees. Even such sprays, which would reduce spore infection, would not assure blotch-free trees as long as blotch-fungus cankers adjoined the inserted buds.

Ordinary nursery workers are not familiar with the appearance or seriousness of the blotch fungus on apple seedlings, as is clearly demonstrated by figure 3, *5*. It will be seen that preparatory to inserting the buds, the budder had made both cuts into active blotch cankers on the seedlings. A satisfactory union of the buds with the seedling stocks had occurred in both cases. On September 7, when the seedlings were collected, the dormant buds appeared normal and healthy. There is small chance of such buds growing into healthy trees, for practically none of the canker tissues would be removed in topping the seedling trees back to the buds in the spring.

The seedlings described and photographed are typical of numerous budded stocks observed scattered over this block of 5,000 lined-out seedlings. In all cases the causal fungus was observed to have grown from the 1927 cankers during the summer of 1929. This indicated that cold storage for a period of nine months does not materially reduce the danger of blotch infection from cankered seedlings.

This ability of *Phyllosticta solitaria* to live in an apparently dormant condition from November or December, 1927, to March or April, 1929, and then renew active growth and spore production indicates the importance of inspecting and culling out blotch-cankered apple seedlings intended for holding in cold storage, the same as advised for seedlings intended for propagation purposes the following year.

SUMMARY

Since 1923 blotch cankers have been found on apple seedlings shipped into Tennessee from midwestern nurseries.

Blotch cankers were found early in March, 1929, on apple seedlings grown in midwestern nurseries in 1927 and held in cold storage throughout the growing season of 1928. There was no evidence that *Phyllosticta solitaria* made any growth during the storage period.

Stored seedlings having numerous blotch cankers, set in large pots in the greenhouse, showed new canker-fungus growth as the season advanced.

Leaves on the stored seedlings and on small seedlings grown directly from seed planted in the pots developed typical blotch leaf spots.

Later in the season typical blotch petiole infections developed on the trees from seed planted in the pots.

Similar cold-storage seedlings in field test blocks developed new canker growth in 1929, enlarging the 1927 cankers.

Trees from the same lot of stored seedlings planted in a commercial nursery about 200 miles from the University farm showed similar new canker development in 1929 from the 1927 cankers.

Observations on the location of inserted buds in relation to blotch cankers indicated that a large percentage of infection results.

The renewed activity of *Phyllosticta solitaria* in 1929, after remaining dormant since the fall of 1927, stresses the importance of inspection and removal of all blotch-infected seedlings from stocks intended for holding in cold storage.

AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF TENNESSEE,
KNOXVILLE, TENNESSEE.

REPORT OF THE FOURTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meetings of the Pacific Division of the American Phytopathological Society were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies, June 19–20, 1930, at the University of Oregon at Eugene.

At the business meeting, which preceded the presentation of papers, the following officers were elected to serve the society for the next two years:

President ..... E. CARSNER, U. C. Citrus Experiment Station, Riverside, California.
Vice-President ..... J. M. RAEDER, University of Idaho, Moscow, Idaho.
Secretary-Treasurer ..... B. A. RUDOLPH, U. C. Deciduous Fruit Station, San Jose, California.
Councillor ..... C. E. OWENS, State College, Corvallis, Oregon.

The attendance of members at the meetings was not so large as hoped for, but those present were enthusiastic and deeply appreciative of the many courtesies extended by their colleagues in the Pacific Northwest. After adjournment the visiting members were taken in automobiles to the State College at Corvallis, a distance of about forty miles. Here an opportunity was afforded to inspect the laboratories and experimental grounds of the Department of Plant Pathology. In the evening a delicious supper was served on the lawn, after which the visiting members motored back to headquarters at Eugene.

There was an average attendance of nineteen members with a varying number of visitors. Twenty-one papers were presented, abstracts of which follow.

B. A. RUDOLPH, *Secretary-Treasurer*

ABSTRACTS

Some inoculations of Juglans with Phytophthora-like fungi.—C. O. SMITH and J. T. BARRETT.

With the use of the California black walnuts as a stock for the English walnut, has appeared the disease known as crown rot. This is an infection of the black walnut crown. Either of two fungi may be the causal organism (1) a species of Phytophthora of the *Phytophthora cactorum* type or (2) a Phytophthora of the Pythiacystis type. The disease may spread to the English walnut trunk and extend upon it for several feet. The walnut Phytophthora has been observed by Barrett producing vegetative growth and oospores on crown-rot bark of English walnut that was wrapped for five days in paraffin paper.

Artificial inoculations on *Juglans californica* (the southern California black walnut) has shown it to be most susceptible. Two hundred fifty-three inoculations gave 80 per cent positive lesions. *Juglans hindsii* (the northern California black walnut), 350 inoculations, gave 50 per cent positive infection. *Juglans regia* (English walnut), 209 inoculations, showed 82 per cent positive infection. The susceptibility of *J. regia* to artificial inoculations, is in marked contrast to its apparent resistance to the crown-rot disease as shown by field observations. The lesions on the English walnut are much smaller than those on *Juglans californica* where, on some mature trees, they reached the size of 60 and 90 inches in a year's time. *Juglans sieboldiana, Juglans nigra, Juglans major* and a Juglans from Mexico (*J. pyriformis*) as well as almonds, peaches, sweet cherry, pear, *Prunus domestica,* and lemon fruits are all susceptible when inoculated. Cultures of *Phytophthora cactorum* from various sources and hosts when inoculated into *Juglans californica* produced lesions typical of walnut crown rot.

It has been difficult to isolate the pathogenes from the diseased crown of *Juglans californica* because of a toxic exudate from the cut tissue. The isolations are more successfully made from the infected English walnut tissue.

A test was made of the toxic effect of one gram of walnut bark boiled in 15 cc. of glucose-potato agar, then plated and inoculated with mycelium of the two walnut pathogenes. The following is the measure of mycelial growth after 20 days in petri dishes:

Diseased southern California black bark and agar	1 mm.
Normal " " " " " "	1.5 cm.
Diseased English bark and agar	6.0 cm.
Normal " " " "	2.25 cm.
Control agar plates covered with growth.	

Relation of cane blight (Leptosphaeria coniothyrium) to lesions of yellow rust (Phragmidium imitans) of red raspberry in Oregon.—S. M. ZELLER.

In a recent article (Jour. Agr. Research 34: 857–863, *illus.* 1927) the writer described the yellow rust of raspberry and pointed out the serious cankerous effects of cane lesions near the ground. Since then much greater losses of canes have been found to be produced by a subsequent infection of these rust lesions by *Leptosphaeria coniothyrium.* The cane blight causes long basal cankers. This combination of the two diseases has caused a loss of 50 to 60 per cent of the canes in the most severe cases, and an estimated annual loss in crop of $75,000 in the Willamette Valley in 1928 and 1929. The control measures suggested are complete sanitation to control basal infections of the canes by the rust. That is, old stubs should be removed and old canes cut so as to leave no stubs, then late fall plowing toward the rows after all the leaves are down.

A blue mold affecting tulips.—F. D. HEALD and KENNETH BAKER.

This trouble was found on one large shipment of bulbs, 60 per cent of them showing lesions involving several of the outer scales, with a Penicillium fruiting abundantly. The relation of the fungus to the rotting of bulbs was determined by pure-culture inoculations on bulbs held in moist chambers and on others planted in the greenhouse. At the end of three weeks the bulbs in the greenhouse showed 40 per cent completely rotted, while the others showed lesions 15–25 mm. in diameter. Those in the moist chambers showed lesions of similar size but no complete rotting. The blue mold continued to spread, involving the entire surface of the bulbs with variable penetration. Inoculations into incisions grew more rapidly, but infections were also obtained by dipping bulbs in a suspension of the blue mold spores, the number of lesions varying from one to six per bulb.

Inoculations of the tulip blue mold in comparison with *P. expansum* were made on tulips, gladioli, and apples. The tulip mold was able to rot apples but not gladiolus bulbs. The rot on apples was much slower than that caused by *P. expansum* and the decayed tissue darker and firmer. When Jonathan apples inoculated with *P. expansum* were completely rotted, the parallel inoculations with the tulip mold showed lesions 3.5–4.5 cm. in diameter.

A witches' broom of ocean spray (Holodiscus discolor Max.).—S. M. ZELLER.

Since 1925 observations of a severe brooming and dwarfing of *Holodiscus discolor* Max. have been made. This disease has been found in the foothills of the western slope of the Cascade Mountains from Linn to Clackamas counties, Oregon. The internodes of the stems are short and stouter than normal. The stems have little tendency to branch so there are usually no blossoms. There is a multiplication of buds at each node. These produce very spindly short laterals. Leaves are small and turn a bronzy red early in the summer. Budding of diseased nodes into healthy stems and the transfer of aphis from diseased to healthy plants have both apparently induced the disease.

Budded branches show the symptoms first and then there is a spread of abnormal growth to other parts of the plant. Insects which might induce such abnormal bud production have not been observed. The symptoms and performance thus far observed would indicate this to be a virus disease.

The effect and the rate of spread of "streak" on greenhouse tomatoes.—LEON K. JONES.

"Streak" of tomato produced by combined viruses of potato and tobacco reduced yields of tomato 50 per cent in an experimental greenhouse planting. Within sixty days after four plants were inoculated in a bed, the remaining sixty-one plants were affected with streak. This spread of the disease was due to cultural and pruning practices, as the plants were free from aphids. Hands were carefully washed with soap and water before pruning in two adjacent control beds of sixty-five plants each. These adjacent beds remained free from streak through the sixty-day period mentioned above. This experiment shows that streak may be spread very rapidly by ordinary pruning methods and that frequent washing of hands with soap and water while pruning reduces the rate of spread of this disease.

Studies of perennial-canker disease of the apple.—J. S. COOLEY and P. W. MILLER.

Successful inoculations of *Gloeosporium perennans* were accomplished throughout the rainy season of 1929 and 1930 or from September to June. The highest percentage of positive infection was in the fall. A study was reported of the susceptibility of wounds made by breaking spurs, by hacking with a saw, by making a smooth incision with a knife, by brushing with a pointed-head mallet, and by driving a small nail into the wood. Those wounds made with a nail and by bruising gave much higher percentages of infection than those made with a sharp tool.

Infection in the inoculation experiments reported was accomplished in fresh wounds that were free from woolly-aphis infestation.

Experiments dealing with the mode of infection under natural conditions of healing calluses was reported. Infection may occur on fresh wounds, but much of it occurs at healing calluses that have been injured by cold. In the summer of 1929 the canker fungus was isolated from pruning-wound calluses having one or more dead areas on the margin that resembled winter injury followed by canker infection. The dead portion of the calluses varied from two to ten or more millimeters in width. In March, following the cold weather in January, 1930, isolations were made from calluses free from

canker in 1929 but showing dead margins that apparently had resulted from recent winter injury. Most of these first attempts at isolating the fungus gave negative results, but the later isolations showed an increasing number of positive infections by the canker fungus. This indicates that the margins of healthy calluses may be injured by cold and that canker infection readily takes place in the winter-injured margins of pruning-wound calluses.

Chemically-treated wraps for the control of Botrytis rot of stored fruits.—J. S. COOLEY.

A rot very prevalent in stored pears and sometimes in apples is caused by Botrytis. The greater part of the loss resulting from this rot is caused by its spread from one fruit to another. The fungus grows through the paper and attacks noninjured fruits The data presented show the efficacy of a chemically-treated wrapper in preventing the spread of this rot.

Pears were wrapped and stored in wraps treated with salts of copper, iron, chromium, nickel, and sodium and also nontreated paper. One layer in each box was inoculated with Botrytis and all fruit was wrapped in treated paper of a given kind. After four or five months of storage, the lot in nontreated wrappers contained only one or two sound fruits to the box while the fruit in the treated wrappers showed that the spread of the fungus was materially affected by the toxic salt in the paper.

A very successful wrapper was prepared by wetting with a 2½% solution of hydrated copper sulphate, the paper absorbing 5.6% by weight of copper sulphate, or 1.4% metallic copper. When the fruit was wrapped in such papers the spread of the Botrytis rot was controlled without chemical injury to the fruit.

The avocado disease called sun-blotch.—WM. T. HORNE and E. R. PARKER.

THE name sun-blotch[1] has been given to a peculiar avocado disease characterized by obscure longitudinal streaks or blotches in the fruit, streaks in vigorous shoots, roughening and decumbency in older stems, and rough bark of trunks. An irregular leaf variegation observed by the writers is believed to be connected with the disease. In green fruit the streaks remain light or yellowish, but in the varieties they become variously red-purple. In its more intense phase the disease is very serious, but symptoms may be slight.

Sun-blotch scions produce sun-blotch trees when set in healthy stocks. Healthy scions in affected stocks probably also produce diseased trees.

In three cases, where diseased scions were set in healthy stocks by the junior author, shoots subsequently arising from the stocks were clearly affected with sun-blotch. Various inoculations and treatment with X-rays have thus far failed to produce the disease.

Apparently sun-blotch is an infectious chlorosis.

A disease of date palm inflorescences.—L. J. KLOTZ and E. C. RABY.

It seems opportune to report at this meeting the recent finding in the Coachella Valley of a disease of date palm inflorescences which may become of serious economic importance. A hasty survey revealed but one infected palm. The disease apparently limits its attack to the fruit stalk and rachises of the young inflorescences. The latter were found diseased even before the spathe had ruptured. On the spathe, circular to elongated lesions, sorghum brown (Ridgway) on the exterior surface and ranging from this color to a mahogany red or bay on the interior surface, marked the starting points

[1] Coit, J. E., Sun-blotch of the avocado, a serious physiological disease. Yearbook Calif. Avocado Ass'n., 1928, pp. 27–29, 5 illus. Los Angeles, Calif., Sept., 1928.

of the disease. On removal of a portion of the infected spathe it was found that the fruit stalk bore depressed, brown (warm blackish) to black necrotic areas, circular to oblong in outline. The twisted deformed rachises were completely involved, blackish brown to black, and devoid of flowers. Microscopic examination showed them covered by dark brown, unicellular, oval conidia. The rachises of fruit bunches attacked later in their development had blackened, depressed lesions similar to those on the fruit stalk, and some were completely severed by the decay. The affected tissue was in all instances dry and firm, and each area bore the black powdery spores. A gray covering on some of the lesions was found due to conidia of *Fusarium* spp.

While the malady has features in common with the Khamedj disease of date palms in Northern Africa, described by Cavara, Chabrolin, and others, the black lesions do not have the white, tomentose covering of the fungus, *Mauginiella scattae* Cav., to which they ascribe the cause of Khamedj. From the interior tissue of the material collected at Indio, a Torulae-like Hyphomycete, *Thielaviopsis* sp., was invariably isolated. On culture media this organism produces a white, aerial growth bearing chains of hyaline conidia which rapidly darken as they mature. Sufficient time has not elapsed since our inoculations to permit us to report on the pathogenicity of this fungus. It may be mentioned that the senior author has found this organism also in connection with the bud scorch and "fool" diseases of date palm.

A graft-infectious disease of the cherry.—T. E. RAWLINS and W. T. HORNE.

A severe disease of the sweet cherry occurs in several valleys in California. Diseased trees produce fruit that is more or less conical in form. The development of such fruit ceases just before ripening, and the fruit hangs on the tree in this immature state for some time, finally shriveling without ripening. During early autumn the leaves on diseased trees may show a peculiar reddish purple coloration along the base of the midrib which extends out along the larger veins. This leaf symptom varies considerably according to the season and may be difficult to detect during certain seasons.

The disease has been transmitted by grafting scions from diseased Napoleon trees into healthy trees.

Trees on Mahaleb roots have shown considerable resistance to the disease, while those on Mazzard roots are very susceptible.

Leaf roll transmission from potato to other solanaceous plants by means of Myzus persicae.—T. P. DYKSTRA.

Leaf roll of potato was transmitted by means of *Myzus persicae* to tomato, pepper, *Datura stramonium*, *Datura tatula*, *Solanum nigrum*, and *Solanum dulcamara*.

Infections varying from 70 to 100 per cent were generally secured. After symptoms became apparent in the plants, aphids were colonized on them, and, after a few days, transferred to potato. In every case a high percentage of leaf roll developed on potato when return inoculations were made from infected plants.

Cross inoculations from Jimson weed to tomato, from tomato to Jimson weed, from *Solanum dulcamara* to tomato, from tomato to tomato, and from Jimson weed to *Solanum nigrum* were also made, and resulted in high percentages of infection.

Symptoms of the two Datura species and of *Solanum nigrum* are characterized by chlorosis and rolling and subsequent rigidity of the leaves.

Leaves of infected tomato plants become stiff and leathery, but this is the only distinguishing symptom, and the disease on this host is somewhat difficult to detect. Pepper plants do not show any marked symptoms until after the disease has been present for several weeks; then the leaves begin to roll and become leathery.

Some experiments in fresh fruit disinfection.—H. P. BARSS.

Four years' tests with disinfectants on fresh prunes and cherries for the suppression of decay during storage or shipping were described. Formaldehyde, used as a dip or three-to-six-minute soak, in strengths of one to one hundred or greater, was found to give marked protection, although the higher concentrations sometimes resulted in skin injury and affected the flavor. Diatomaceous earth, impregnated with four to five per cent of formaldehyde and dusted over the fruit, gave excellent control but affected the skin somewhat. The most practical protection was obtained through the use of a sheet of absorbent paper impregnated with formaldehyde placed in the bottom of the box of fruit and another in the top. When the dose was not too strong, decay was suppressed more effectively than by the liquid treatments and without perceptible injury to flavor, color, or luster. Thus far the latter method has not been tested on prunes. Borax alone and with boric acid in solution, acetic acid, hexylresorcinol, and a commercial preparation yielding free chlorine proved inferior or useless. In general, washing often reduced the effectiveness of liquid treatments.

Didymosphaeria oregonensis, *a new canker organism on alder.*—L. N. GOODDING.

This paper describes as new a species of Didymosphaeria found associated with a canker on living alder in the Pacific Northwest. The name **Didymosphaeria oregonensis** Goodding is proposed. The distribution of the new species is given as western Oregon and Washington. There also is included a brief consideration of its taxonomic relationship and economic importance.

Cladosporium species from apple fruit and the perfect stage of Cladosporium herbarum LK.—GEORGE D. RUEHLE.

In connection with a study of the fungi causing decay of apples in cold storage, three species of Cladosporium were found to be weak parasites of the apple.

The first produces rapidly growing fluffy colonies and light brown cylindric conidia in long chains. It is considered to be a new species.

The second produces dense slow growing colonies and conidia typical of the form species, *C. herbarum*. When grown on mature sterilized wheat leaves for six months at 10° C., this form produced large numbers of black fertile perithecia of the Mycosphaerella type. On germination, single ascospores produced colonies of the *C. herbarum* type. The ascigerous stage is believed to be identical with *Mycosphaerella tulasnei* described in 1893 by Janczewski as the perfect stage of *C. herbarum*. Positive confirmation of Janczewski's report has been lacking until now.

The third form was identified as *Homodendron cladosporioides* Sacc. and produces no perithecia.

As a group these forms are of little importance on stored apples, although of frequent occurrence on this host. They produce small, shallow dark rots around stem punctures and worm holes but, otherwise, do little damage.

In contrast with seedling stock, apparently healthy potato tubers are virus carriers.—GROVER BURNETT and LEON K. JONES.

Tomato plants were inoculated with macerated leaf tissue of commercial potato-tuber stock and with macerated leaf tissue of potato seedlings, each combined with macerated leaf tissue of tobacco plants affected with common tobacco mosaic.

Plants produced from eighty-eight apparently healthy commercial tubers and thirty-two known to be virus-infected, representing eight commercial varieties, were used in the tests. Each virus-infected tuber showed symptoms of one of the following virus dis-

eases: crinkle, crinkle mosaic, leaf roll, mild mosaic, rugose mosaic, unmottled curly dwarf, witches' broom, super-mild mosaic, and spindle tuber.

"Streak" of tomato developed in every case when macerated tissue from these plants was mixed with macerated tissue of tobacco plants affected with common tobacco mosaic and this juice rubbed onto healthy tomato plants.

In no case did streak develop on tomato plants inoculated with macerated potato-seedling tissue combined with macerated mosaic-tobacco tissue. Forty-seven potato seedlings were used in inoculating 245 tomato plants. Series of control plants were alternated with series of inoculated plants. The 438 plants used as controls remained healthy during the tests.

These results tend to show that potato seedlings do not carry this virus which may be present in all commercial tuber stock.

European and American brown-rot blossom blight in western Oregon.—H. P. BARSS.

Serious blossom blight in cherries occurred in many orchards in the Willamette Valley in 1929 and a considerable amount appeared again in 1930. Isolations from material obtained from widely scattered orchards showed that, while both forms of brown rot, prevalent on the Pacific Coast, were present in the blighted blossoms, the European form, *Sclerotinia cinerea* (Bon.) Schröt. forma *pruni* Wormald, predominated by three to one in 1929 over the common American form, *Sclerotinia fruiticola* (Wint.) Rehm (= *Sclerotinia americana* Nort. et Ezek.), and in 1930 greatly exceeded the latter. From green cherries and prunes, attacked shortly after the shucks fell, only the American form was isolated in the same year. Attack by the European brown rot was reported on sweet-cherry foliage, flowering-almond twigs and cherry leaves previously invaded by the witches' broom, Exoascus.

Measuring duration of effectiveness of Bordeaux sprays to perennial canker spores.—J. H. CRENSHAW.

A method is described for determining how long Bordeaux sprays remain effective in preventing germination of perennial canker spores under ordinary orchard conditions.

Trees were sprayed at intervals throughout the rainy season. After exposure to weathering for different periods, twigs were removed for study. These were inoculated with spore suspensions and the spores given an opportunity to germinate on the twigs. They were then transferred to glass slides for microscopic examination.

Some preliminary measurements were made. They indicate that sprays applied late in December were effective in March, but that sprays applied as late in the fall as October 29th were ineffective before February 22nd of the following year.

Electrophoresis of tobacco-mosaic virus.—WILLIAM N. TAKAHASHI and T. E. RAWLINS.

Tobacco-mosaic virus was subjected to electrophoresis at various pH values. The virus moves toward the anode at pH 4 to 9. No movement was detected between pH 1.2 and 3. The electrophoretic behavior of the virus was similar to that observed for bacteria by other workers.

Some pathological changes induced in citrus by a deficiency of boron.—A. R. C. HAAS and L. J. KLOTZ.

In sand and water cultures lacking boron, several species of Citrus showed the following symptoms of decline: Leaves curled along the midrib with the tip of the leaf bending downward and inward; leaves brownish or yellowish green, often with a yel-

lowing along the midrib; midrib or veins conspicuous, corky and split; and a progressive loss of affected leaves. In severe cases there is a tendency toward "multiple bud" formation due to incipient shoots dying when barely visible. This and other symptoms are similar to those found in exanthema. When the bark of the internodes of the twigs, or in severe cases that of the trunk, splits, an amber-color gum oozes out. Eventually the cracks may widen so that the woody tissue is exposed. In severe cases the apical portion of the branch dies back. The roots become dark brown, fail to elongate, and in advanced cases the rootlets decay. On the addition of a suitable concentration of boron to the culture solution the symptoms of decline disappear and normal growth resumes.

Anatomical studies show that boron is essential for cell division in the meristematic tissue of the growing points, such as buds, and it is likewise essential for cambial activity.

The abnormal accumulation of carbohydrates in the leaves of boron-deficient Citrus, coupled with the fact that the phloem tissues are destroyed, would show that translocation is seriously interfered with. Reduction in the total sugar content of the leaves accompanies recovery of the tissues brought about by the addition of boron to the culture solution.

Some experiments with crown-gall bacteria.—F. A. PATTY.

When plants of tobacco, castor bean, geranium, and garden bean were sprayed with suspensions of crown-gall bacteria, the organisms entered the leaves through natural openings, as indicated by the recovery of virulent organisms from the mesophyll of the leaves after stripping away the epidermis. Except for the consistent recovery by culture of the crown-gall organism from within sprayed leaves no other indication of the presence of the bacteria appeared, for there was no gall formation or other indication of disturbance in the development of the leaf tissues, even when partially developed leaves were inoculated by this method and held under observation for four weeks. Puncture inoculations with the same strains of the crown-gall organism produced visible enlargements on the stems within six days.

On the control of bacterial blight of walnuts.—B. A. RUDOLPH.

Bacterial blight is by far the worst disease with which walnut growers must contend. During the past three years extensive spraying experiments have been conducted. Virtually all of the better-known chemicals and bactericides have been employed, many of which are harmless to other plants but very injurious to the walnut. Three copper-containing compounds, Bordeaux mixture, basic copper acetate, and ammoniacal copper carbonate, have proved effective in the control of the disease and, incidentally, occasioned distinctly less injury to the trees than most other materials. Bordeaux 8–4–50 (the lime used reduced to one-half) has given the best results of all. The strategic period for securing maximum results with one application of spray is when the catkins are fully elongated and about to shed pollen. The increase in crop on trees sprayed at this time has more than justified the expense of spraying in many instances. In badly diseased orchards or in orchards situated in localities where climatic conditions favor ready infection and the rapid spread of the disease, multiple spraying must be employed. The first spray is given as before, and subsequent sprays follow several months apart to protect the growing nuts which are susceptible at any time until the harvest in October. Thoroughness of application of the spray cannot be stressed too much.